49028-88

Wastewater and Biosolids Analysis Manual

Digestion and Selected Methods for Determining Metals, Minerals, and Other Related Parameters

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SECTION 1 INTRODUCTION

This comprehensive biosolids handbook provides wastewater treatment personnel with a step-by-step guide to cost-effective in-house testing methods. The methods detect and quantify nutrients (potassium, nitrogen...) and metals (lead, cadmium...) as well as measure solids in wastewater liquids and biosolids.

The methods are simple and time-efficient. Each step is illustrated in an easy-to-use format. Lists of apparatus and reagents to perform the analysis are at the end of each method.

Also included are step-by-step digestion methods for liquids and solids using Hach's Digesdahl® Apparatus. Using the Digesdahl will save time and make it easy to use the digestate for analysis. A decision tree helps to select the correct type of digestion.

Results from these methods can be used to improve biosolids reclamation, protect against application of high levels of contaminants, and ensure safe biosolid disposal.

This manual is divided into six sections:

- Section 2 Guide to Using Instruments – has step-by-step instructions for using Hach TenSette® Pipets and spectrophotometers required in the procedures, as well as information on adapting other spectrophotometers for use with the procedures.

- Section 3 Digestion Information – provides instructions for two USEPA-approved digestions for use when results are reported to regulatory agencies. This section also contains two Digesdahl digestion procedures, one for aqueous solutions and one for solids. Be sure to read the safety information provided before using the Digesdahl.

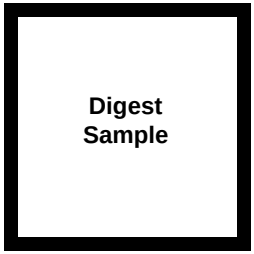
- Section 4 Analysis Methods – contains over 40 methods for determining 27 parameters in influent, primary influent, secondary influent, and sludge. These procedures use precalibrated spectrophotometers and colorimeters for direct colorimetric measurement (except for solids determinations).

- Section 5 General Information – includes information on how to order items, repair service, and warranty.

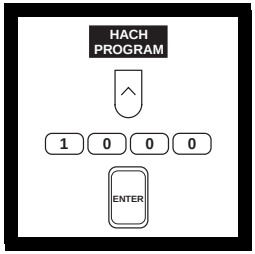
- Appendix – has troubleshooting, USEPA sampling and storage information, and a list of parameters that each Hach spectrophotometer determines, as well as the test range.

2.1 Using the DR/4000 Spectrophotometer

Typical Procedure

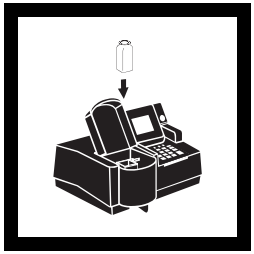


1. Prepare the sample digest for measurement as described in the applicable test procedure. If the reagent blank needs treatment, prepare it at this time.

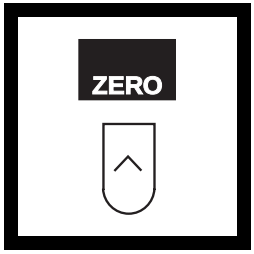


2. Select the desired stored program by pressing the soft key under ***HACH PROGRAM***. Enter the program number by using the numeric keys. Press **ENTER**.

3. The display will show the program name and number. The wavelength will be automatically selected.

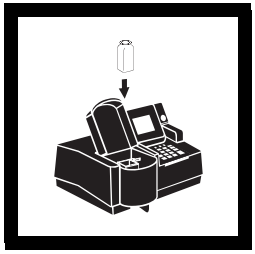


4. Place the reagent blank into the cell holder and close the light shield.



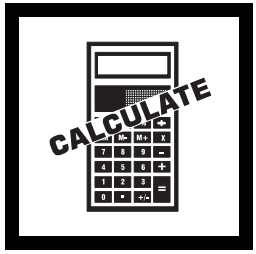
5. Press the soft key under ***ZERO***.

The display will show a zero value, measurement unit, and the parameter.



6. Place the prepared test sample into the cell holder and close the light shield.

The result will be displayed in the chosen unit of measure.



7. To compensate for sample dilution during digestion, calculate the true concentration using the equation under the Sample Analysis and Sample Volume tables following each procedure.

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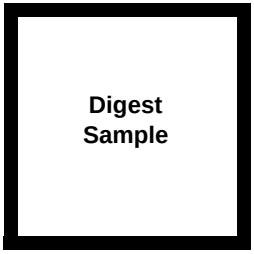
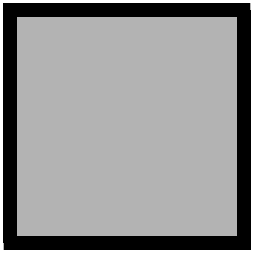
2.2 Using the DR/2010 Spectrophotometer

Typical Method

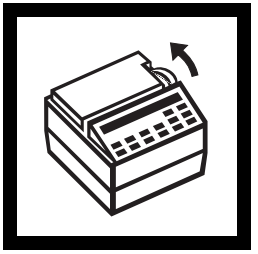


1. Prepare the sample digest for measurement as described in the applicable test procedure. If the reagent blank needs treatment, prepare it at this time.

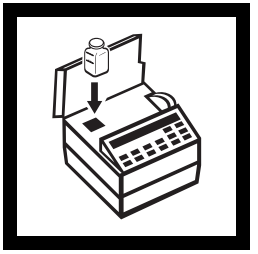


2. Using the numeric keys, enter the stored program number for the desired parameter. Press **ENTER**.

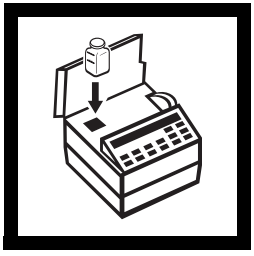
The display will prompt users to dial to the correct wavelength.



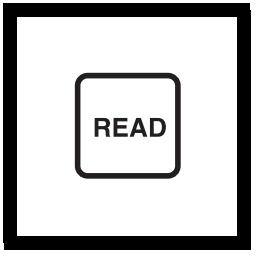
3. Rotate the wavelength dial to the value specified in the display. The display will show **Zero Sample**, then the measurement unit and parameter.



4. Place the reagent blank into the cell holder and close the light shield. Press **ZERO**. The display will show **Zeroing...**, then a zero value, measurement unit, and the parameter.

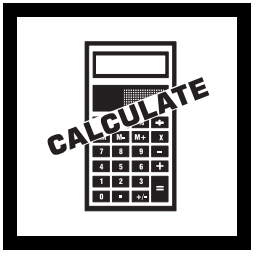


5. Place the prepared test sample into the cell holder and close the light shield.



6. Press: **READ**

The display will show **Reading...**, then the result will be displayed.



7. To compensate for sample dilution during digestion, calculate the true concentration using the equation under the Sample Analysis and Sample Volume tables following each procedure.

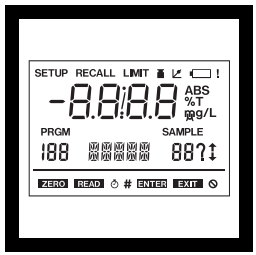
2.3 Using the DR/800 Colorimeter

Typical Method

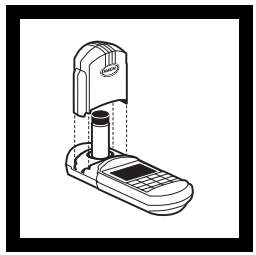
1. Prepare the sample digest for measurement as described in the applicable test procedure. If the reagent blank needs treatment, prepare it at this time.

2. Press **PRGM**

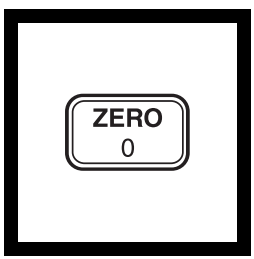
The display will show **PRGM ?**. Using the numeric keys, enter the stored program number for the desired parameter.



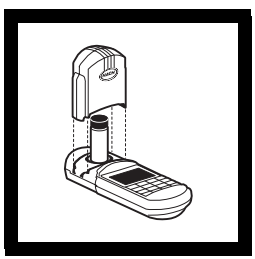
3. The display will show measurement units, the parameter, and the **ZERO** icon.



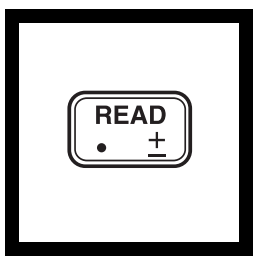
4. Place the reagent blank into the cell holder. Tightly cover the sample cell with the instrument cap.



5. Press **ZERO**. The cursor will move to the right then the display will show a zero value, measurement unit, and the parameter.

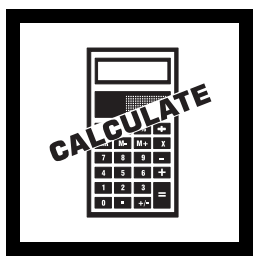


6. Place the prepared test sample into the cell holder. Tightly cover the sample cell with the instrument cap.



7. Press: **READ**

The cursor will move to the right, then the result will be displayed.



8. To compensate for sample dilution during digestion, calculate the true concentration using the equation under the Sample Analysis and Sample Volume tables following each procedure.

2.4 Adapting Hach Methods to Other Spectrophotometers

Hach test methods can be used with other instruments by creating a calibration curve to convert percent transmittance or absorbance readings to concentration (mg/L, µg/L...). Regardless of the instrument used, the sample and standardizing solutions are prepared the same way and the optimum wavelength specified in a Hach method applies to other spectrophotometers.

The example below describes a calibration for iron in the 0 to 2.4 mg/L range.

1. Prepare a series of five or more iron standard solutions that cover the expected range of iron in the sample. Run the procedure with the standards as described in the step-by-step instructions.

2. Pour the customary volume of each standard solution into separate, clean sample cells used by the instrument.

3. Select the proper wavelength (or color filter) and standardize the instrument using untreated sample or a reagent blank as specified in the procedure.

4. Mix the reagents and sample in the manner specified in the step-by-step instructions.

5. Measure the blank and standards. Plot the readings on graph paper (see *Figure 1*), with the absorbance on the vertical (y) axis and the concentration on the horizontal (x) axis. In this example, iron standards of 0.1, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 mg/L were measured at 500 nm.

Note: *Percent transmittance vs. concentration can be plotted on semilogarithmic graph paper to obtain a calibration curve.*

6. Plot the calibration data points on the graph. Draw a line that connects the points. This is the calibration curve.

7. The calibration curve is all that is necessary to determine sample concentrations. It may be easier to use a calibration table (see *Table 1*), which can be constructed by extrapolating from the curve. One may also use the formula for the best-fit line:

   concentration = x-axis intercept + slope x abs

In this case (see *Figure 1*):

concentration (mg/L) = 0 + (2.294 x abs)

Example: 0 + (2.294 x 0.34) = 0.779 mg/L

Figure 1 Calibration Curve Example

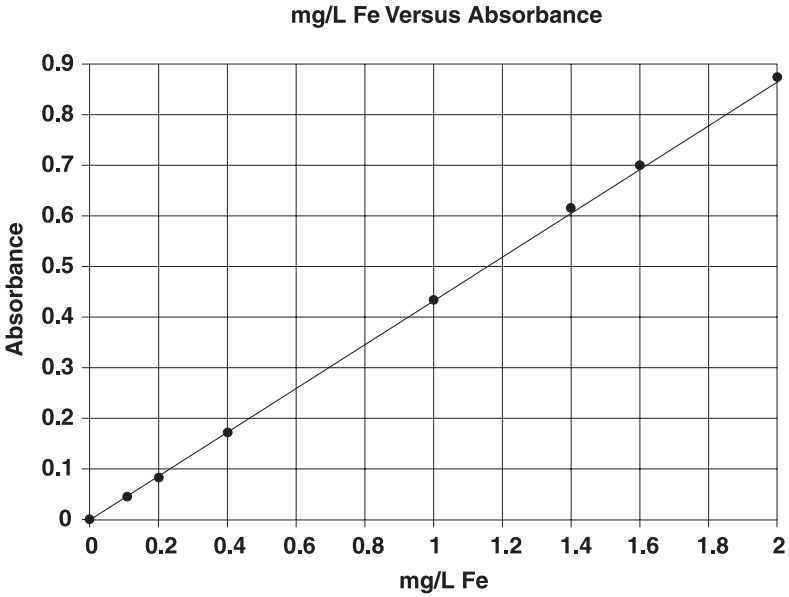


Table 1 Calibration Table Example

	Iron Concentration (mg/L)										
Abs Units	**0.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
0.00	0.00	0.23	0.46	0.69	0.92	1.15	1.38	1.61	1.83	2,06	2.29
0.01	0.02	0.25	0.48	0.71	0.94	1.17	1.40	1.63	1.85	2.08	2.31
0.02	0.05	0.28	0.50	0.73	0.96	1.19	1.42	1.65	1.88	2.11	2.34
0.03	0.07	0.30	0.53	0.76	0.99	1.22	1.45	1.67	1.90	2.13	2.36
0.04	0.09	0.32	0.55	0.78	1.01	1.24	1.47	1.70	1.93	2.16	2.39
0.05	0.11	0.34	0.57	0.80	1.03	1.26	1.49	1.72	1.95	2.18	1.41
0.06	0.14	0.37	0.60	0.83	1.06	1.28	1.51	1.74	1.97	2.20	2.43
0.07	0.16	0.39	0.62	0.85	1.08	1.31	1.54	1.77	2.00	2.25	2.48
0.08	0.18	0.41	0.64	0.87	1.10	1.32	1.56	1.79	2.02	2.25	2.48
0.09	0.21	0.44	0.67	0.89	1.12	1.34	1.58	1.81	2.04	2.27	2.50

To convert an absorbance reading to mg/L iron, select the appropriate column from the Iron Concentration column and the appropriate row from the Absorbance Unit column. For example, if the instrument read 0.34, use the “0.3”column and the “0.04” row. The result is 0.78 mg/L iron. An absorbance reading of 0.58 would yield 1.32 mg/L iron.

2.5 Using the TenSette® Pipet

For best results, always use a new tip for each pipetting operation. After several uses, the tip may retain some liquid, causing an error in the delivery volume. Each pipet is supplied with 100 tips. Order Hach replacement tips for best results.

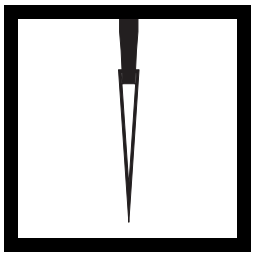
Always use careful, even hand movements to improve reproducibility. If the pipet does not operate smoothly, disassemble and coat the piston and retainer with high-quality stopcock grease. Also, lightly coat the metering turret.

For greatest accuracy, both the room temperature and the temperature of the solution being pipetted should be 20 to 25 °C.

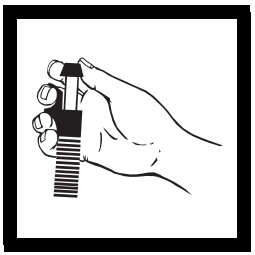
Holding the pipet for extended periods may cause inaccurate volumes due to the transfer of body heat.

Never lay the pipet down with liquid in the tip. Solution could leak into the pipet and cause corrosion.

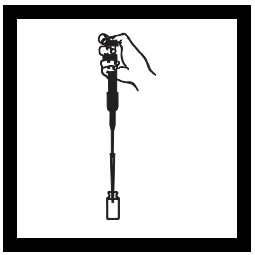
Use of the TenSette Pipet



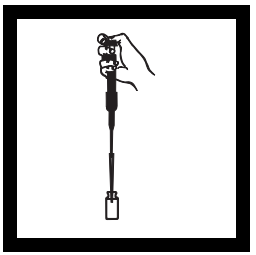
1. Attach a clean tip. Hold the TenSette Pipet in one hand and gently press the tip until it is firmly held and a good seal is formed.



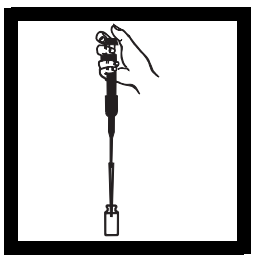
2. Turn the turret cap to align the desired volume with the mark on the housing assembly.



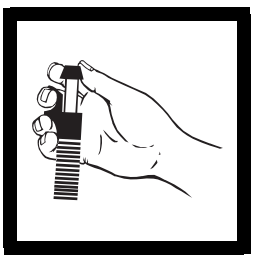
3. Using a smooth motion, press down on the turret cap with the thumb until the turret reaches the stop. Immerse the tip about 5 mm (¼ in.) below the surface of the solution to avoid drawing air into the tip. Do not insert the tip any deeper or the delivery volume may be affected.



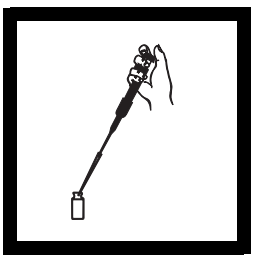
4. Maintaining a constant pressure, allow the turret to return to the extended position. Do not let the turret snap into place—this may affect delivery volume.



5. After the turret returns to the extended position, remove the tip from the liquid and move it to the receiving vessel. Do not place pressure on the cap while moving the pipet.



6. Use the thumb and forefinger to twist the turret cap to the next highest volume to assure full liquid blowout and quantitative transfer of the liquid. The "F" position also provides full blowout.



7. Place the tip in contact with the side of the receiving vessel. Slowly and smoothly press down on the turret cap until the turret reaches the stop and the liquid is completely discharged.

SECTION 3 DIGESTION INFORMATION

3.1 General Digestion Procedures

Several procedures require sample digestion. Digestion uses chemicals and heat to break down a substance into components that can be analyzed. This section has three different digestion procedures.

The Hach Digesdahl® system is a process that yields a digest suitable for the determination of metals, total phosphorus and total kjeldahl nitrogen (TKN). It is rapid, convenient, and the method of choice.

For USEPA reporting purposes, USEPA-approved digestions are required. USEPA presents two digestions (mild and vigorous) for metals analysis. These are much more inconvenient and time consuming compared to the Hach Digesdahl system. Other digestion procedures are required for phosphorus and TKN.

3.1.1 EPA Mild Digestion with Hot Plate for Metals Analysis Only

1. Acidify the entire sample at the time of collection with concentrated nitric acid by adding 5 mL of acid per liter (or quart) of sample.

2. Transfer 100 mL of well-mixed sample to a beaker or flask. Add 5 mL of distilled 1:1 hydrochloric acid (HCl).

3. Heat using a steam bath or hot plate until the volume has been reduced to 15-20 mL. Make certain the sample does not boil.

4. After this treatment, the sample may be filtered to remove any insoluble material.

5. Adjust the digested sample to pH 4 by drop-wise addition of 5.0 N Sodium Hydroxide Standard Solution. Mix thoroughly and check the pH after each addition.

6. Quantitatively transfer the sample with deionized water to a 100-mL volumetric flask and dilute to volume with deionized water. Continue with the procedure. This mild digestion may not suffice for all sample types. A reagent blank also should be carried through the digestion and measurement procedures.

3.1.2 EPA Vigorous Digestion with Hot Plate for Metals Analysis Only

Use a vigorous digestion to ensure all of the organo-metallic bonds are broken.

1. Acidify the entire sample with redistilled 1:1 Nitric Acid Solution to a pH of less than two. Do not filter the sample before digestion.

2. Transfer an appropriate sample volume (see *Table 2*) into a beaker and add 3 mL of concentrated redistilled nitric acid.

3. Place the beaker on a hot plate and evaporate to near dryness, making certain the sample does not boil.

4. Cool the beaker and add another 3 mL of the concentrated redistilled nitric acid.

5. Cover the beaker with a watch glass and return it to the hot plate. Increase the temperature of the hot plate so that a gentle reflux occurs. Add additional acid, if necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change color or appearance with continued refluxing).

6. Again, evaporate to near dryness (do not bake) and cool the beaker. If any residue or precipitate results from the evaporation, add redistilled 1:1 hydrochloric acid (5 mL per 100 mL of final volume). See *Table 2*.

7. Warm the beaker. Add 5 mL of 5.0 N sodium hydroxide and quantitatively transfer the sample with deionized water to a volumetric flask. See *Table 2* below for the suggested final volume.

8. Adjust the sample to pH 4 by drop-wise addition of 5.0 N Sodium Hydroxide Standard Solution; mix thoroughly and check the pH after each addition. Dilute to volume with deionized water. Multiply the result by the correction factor in *Table 2*. A reagent blank also should be carried through the digestion and measurement procedures.

Table 2 Vigorous Digestion Volumes

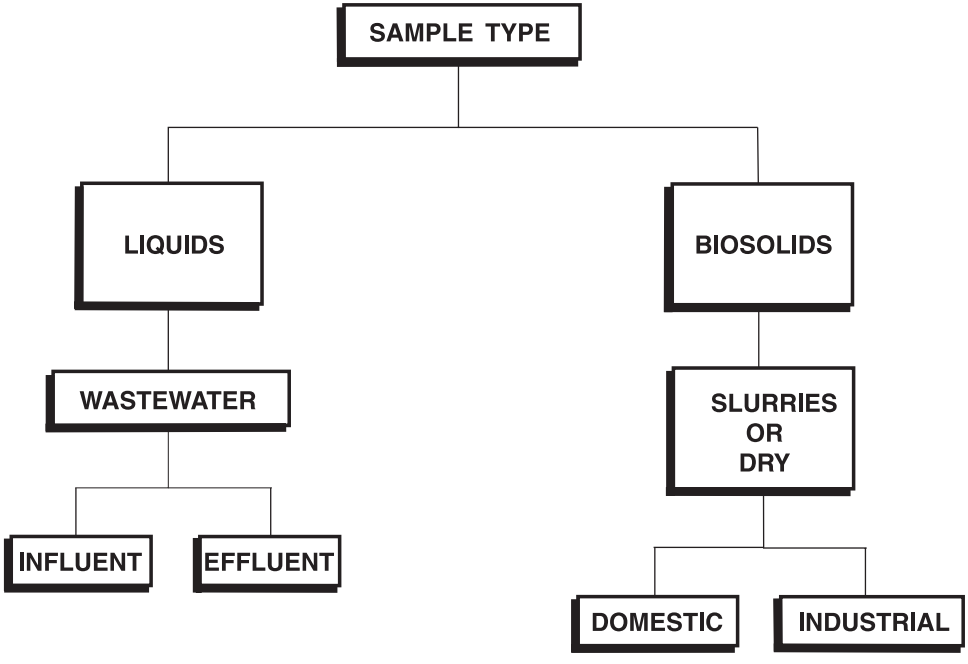
Expected Metal Concentration	Suggested Sample Vol. for Digestion	Suggested Volume of 1:1 HCl	Suggested Final Volume After Digestion	Correction Factor
1 mg/L	50 mL	10 mL	200 mL	4
10 mg/L	5 mL	10 mL	200 mL	40
100 mg/L	1 mL	25 mL	500 mL	500

3.2 Digesdahl Digestion Information

Although the Digesdahl can digest many types of samples, a specific digestion is required for specific sample types. To classify the sample as a liquid or solid, use the following chart (see *Figure 2*). Follow the appropriate procedure indicated for each sample type.

Sample size varies, depending on the sample type and the parameter being measured. Once the correct sample type is decided, use the tables following each of the digestion procedures to determine the sample size.

Figure 2 Sample Types



3.3 Digesdahl Digestion

Important: Please read this section and *Section 3.4 on page 28* before using this apparatus. Read the Material Safety Data Sheet (MSDS) for all reagents.

DANGER
Wear protective eye glasses and clothing. A strong acid (concentrated sulfuric acid) and a strong oxidant (50% hydrogen peroxide) are used in the digestion reaction. These chemicals can cause burns if splashed on the skin or permanent eye damage if allowed to contact the eyes. If the chemicals are hot, effects are considerably more severe. Immediately rinse any affected area thoroughly with water and contact a physician.

Procedures for using the Digesdahl Digestion Apparatus vary with sample type. Most procedures use a two-phase digestion process involving concentrated sulfuric acid and 50% hydrogen peroxide. Sulfuric acid dehydrates and chars the sample. Hydrogen peroxide is added via the capillary flow funnel to complete sample decomposition. The capillary funnel feeds hydrogen peroxide into the digestion flask at a rate of 3 mL per minute. This allows the analyst to control the amount of time sample is exposed to the hydrogen peroxide (digestion time) by varying the volume of hydrogen peroxide used.

Use the Digesdahl Digestion Apparatus only behind a laboratory safety shield or in a closed fume hood.

Some samples are more difficult to digest completely. In a careful study of the minimal time required to digest a variety of materials, complete nitrogen recovery was achieved for many samples immediately upon clearing of the digest (when the digest becomes colorless). Resistant or refractory materials such as nicotinic acid require several minutes of continued peroxide digestion after clearing to obtain 100% nitrogen recovery.

To ensure complete sample digestion, consider the variables described in the following paragraphs.

3.3.1 Appropriate Sample Size

For solid or organic liquid samples, less than 0.5 grams of anhydrous material can usually be digested effectively. As a routine practice, 0.25 g of sample is used. Samples that contain water may be scaled up by a proportional amount. There is no restriction or minimum sample size. For samples of aqueous solutions or suspensions, the maximum volume is 40 mL. When the percent solids exceeds 1% of the sample volume, the maximum sample volume should be reduced using the formula:

$$\text{sample} = \frac{40\ \text{mL}}{\%\ \text{solids}}$$

3.3.2 Proper Digestion Solution Temperature

Digestion temperature is critical. If the hydrogen peroxide is added to a cold digestion mixture followed by heating, the hydrogen peroxide decomposes before the digest reaches the proper temperature. Adding hydrogen peroxide to a digest that is too hot volatilizes most of the oxidant with little benefit. Also, excessive heat contributes to spray loss of sample as a fine mist. The temperature recommended for most samples is 440 °C (825 °F).

3.3.3 Sufficient Sulfuric Acid

The amount of concentrated (specific gravity 1.84) sulfuric acid used **must be** sufficient to prevent the digestion from going to dryness. Any portion of the flask bottom that becomes dry will overheat and may cause the flask to explode. Also, too little acid will cause the sample to overheat, which may cause thermal decomposition of desired analytes (i.e., ammonium compounds) and result in sample loss. **Use an amount of sulfuric acid that will leave at least 2 mL of residual acid when digestion is complete**. Refer to *Table 3* for recommended volumes.

Sulfuric acid (H_2SO_4) consumption depends on the anhydrous mass of material and the chemical composition of the substance. Use of 4 mL H_2SO_4 is suitable for many materials, but not all. Therefore, the analyst must pay attention to the amount of residual H_2SO_4 for the type of sample digested, and adjust the amount of acid or sample accordingly. Never use less than 3 mL of concentrated sulfuric acid. Larger volumes of H_2SO_4 may be used, but avoid a large excess since sample pH adjustment is required in most subsequent determinations.

Table 3 Digestion Guidelines of Specific Sample Types

Sample Type	Sample Weight	Vol. of Acid	Preheat Time (Step 5)	Vol. of Peroxide	Special Instructions
Plant tissue	0.25 to 0.5 g	4 mL	4 min.	10 mL	Use Nitrogen-free paper to weigh samples.
Meat & Poultry	0.5 g or predigestion	4 mL or as in predigest	4 min.	10 mL	—
Fluid Fertilizers	0.1 to 0.25 g	4 mL	4 min.	10 mL	Add 0.4 g Kjeldahl Reduction Powder (Cat. No. 23653-04) to flask before adding sulfuric acid. Place the flask in an 80 °C oven 15 minutes before digestion. Use N-free paper to weigh samples.
Feed & Forage	0.25 g	4 mL	4 min.	10 mL	—
Dairy	0.25 to 2.0 g	4 mL	4 min.	10 mL	—
Cereal	0.25 to 0.5 g	4 mL	4 min.	10 mL	Use Nitrogen-free paper to weigh samples.
Beverage	about 5 g (pipet into funnel)	4 mL	1 min.	10 mL	Preheat acid for 1 minute then add sample through funnel. Heat flask for 30 seconds after sample is in the flask.
Sludge	<2.5 g wet sludge <0.5 g dried sludge	4 mL	3-5 min.	10 mL or increase in 5 mL increments	Heat the diluted digest for 15 minutes and filter.
Water & Wastewater	not more than 0.5 g solid (mL = 40/C; C = % solids)	3 mL	until acid is refluxing	10 mL or increase in 5 mL increments	Water must evaporate before acid will reflux. **Boiling chips required.**
Bath Solutions	0.3 to 10 mL	4 mL	4 min.	10 mL	Water must evaporate before acid will reflux. **Boiling chips required.**
Edible Oils	0.25 to 5.0 g	4-6 mL	4 min.	5 mL immediately and 5 mL later	Weigh samples into flask and record exact weight.
Ion Exchange Resins	equivalent of 0.25 g dry resin	10-15 mL	12 min.	20 mL	Digest will be clear with particles on bottom if metal oxides are not soluble in H_2SO_4. Add aqua reagia or suitable solvent to dissolve particles. If particles are floating, start again using 15 mL H_2SO_4 and longer char time.
Soil	0.25 to 0.5 g	6 mL	4 min.	10-20 mL	—
Fuels	0.25 to 0.5 g	6 mL	4 min.	20 mL	Heat the diluted digest for 15 min. and filter. Lower heater temperature if foaming or burning occurs.

3.3.4 Carbonization Period

A carbonization period prior to the addition of hydrogen peroxide provides a reducing environment which helps convert organic nitrogen to ammonia. In the presence of oxidizable carbon compounds, sulfuric acid reacts to produce sulfur dioxide, which is the active reducing agent.

The reaction is:

$H_2SO_4 \rightarrow H_2O + SO_2 + 1/2\ O_2$

A preheat period of 2 to 5 minutes is recommended for routine digestions.

3.3.5 Adequate Peroxide Concentration for Sufficient Time

Researchers believe that hydrogen peroxide (H_2O_2) reacts immediately with H_2SO_4 at digestion temperature to give H_2SO_5 (peroxymonosulfuric acid) by the reaction:

$H_2SO_4 + H_2O_2 \rightarrow H_2SO_5 + H_2O$

This is an extremely powerful **oxidizing agent** toward carbonaceous material. The objective is to maintain an adequate concentration of H_2SO_5 in the hot digestion mixture for a long enough time to complete oxidation of the carbonaceous material. The H_2O_2 is metered into the flask at 3 mL/min. using the capillary funnel.

The amount of peroxide that must be added for complete digestion can be determined by digesting a sample multiple times with incremental increases in the amount of peroxide added (i.e., 5 mL, 10 mL, 15 mL, 20 mL). Graph the results of the analysis for the parameter of interest to determine the minimum amount of peroxide needed for optimum sample digestion.

The preferred and recommended peroxide reagent is 50% hydrogen peroxide. Research studies have shown that the best recovery and reproducibility are achieved using the 50% peroxide reagent. The 50% hydrogen peroxide reagent produces dependable results in research and actual applications. The optimal rate of peroxide addition has been determined as 3 mL/minute using the 50% reagent. This may not hold true if other strengths of hydrogen peroxide are used.

If 50% hydrogen peroxide is not available, 30% peroxide may be used as a last resort with caution. Because of the lesser strength, at least 1.67 times more volume must be used (i.e., 16.7 mL of 30% vs. 10 mL of 50% peroxide). Always run a digestion standard, either glycine p-toluenesulfonate or nicotinic acid p toluenesulfonate, when using 30% peroxide to check

completion of the digestion. See *Section 3.3.8* on page *27*. All recommended safety precautions apply to both strengths of the hydrogen peroxide. See *Digesdahl Digestion Safety* on page *28.*

3.3.6 Containment of Sample

Loss of sample from the digestion flask can occur in two ways:

(1) foaming or boiling over, and

(2) mist or spray in the ventilation air stream.

Foaming is a serious problem with certain sample types, so special techniques have been developed. Some liquid samples, especially those containing sugars, cannot be digested by the standard procedure, which consists of placing a given volume in the flask, adding sulfuric acid and heating. Under those conditions, foaming cannot be controlled. Instead, the sulfuric acid is heated in the flask and the liquid sample is added through the capillary funnel. Carbonization proceeds in a controlled manner. When all the sample has been added, the peroxide treatment begins and the digestion continues normally. Other ways to control foaming include early addition of hydrogen peroxide and reducing the temperature during carbonization.

Sample loss as spray or mist occurs when small droplets of liquid are swept out of the flask along with gases, and escape in the ventilation air stream. The current fractionating column design reduces spray loss to an insignificant level.

3.3.7 Sampling and Storage

Samples must be homogeneous to ensure that a representative portion is analyzed. Liquid samples should be homogenized via stirring or blending. Solid samples should be finely ground or chopped and well mixed. After digestion, samples should be diluted to the mark, mixed, and tightly sealed. The diluted digestate will be stable for 2 to 3 days, as long as evaporation does not occur.

3.3.8 Accuracy Check

Complete digestion is necessary for accurate results. The Digesdahl system may be checked with Primary Standards for Kjeldahl Nitrogen using one of the following methods.

Solid Samples

1. Weigh 0.25 grams of a Primary Standard for Kjeldahl Nitrogen. See *Table 4*. Digest the standard following the general digestion procedure.

2. Any of the three standards may be used. Ammonium p-toluenesulfonate (185.5 mg/L TKN) is the least difficult to digest. Glycine p-toluenesulfonate (141.63 mg/L TKN) is moderately difficult to digest, and Nicotinic Acid p-toluenesulfonate (118.58 mg/L TKN) is the most difficult to digest.

Liquid Samples

1. Weigh 10.000 grams of a Primary Standard for Kjeldahl Nitrogen. See *Table 4*.

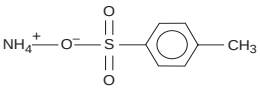
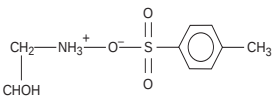
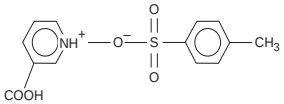
2. Transfer to a 1-liter volumetric flask and dilute to the mark.

3. Using a volumetric pipet, add 15 mL of the prepared solution to the digestion flask and digest the standard following the general digestion procedure.

4. Any of the three standards may be used. Ammonium p-toluenesulfonate (185.5 mg/L TKN) is the least difficult to digest. Glycine p-toluenesulfonate (141.63 mg/L TKN) is moderately difficult to digest, and Nicotinic Acid p-toluenesulfonate (118.58 mg/L TKN) is the most difficult to digest.

A primary standard set, containing one bottle of each of the standards, may be purchased from Hach Company. *Table 4* lists some of the properties of the three primary standards.

Table 4 Some Properties of Kjeldahl Nitrogen Standards (#22778-00)

	Ammonia PTSA	Glycine PTSA	Nicotinic Acid PTSA
Formula	$C_7H_{11}O_3SN$	$C_9H_{13}O_5SN$	$C_{13}H_{13}O_5SN$
Structure			
Molecular Weight	189.235	247.277	295.316
Melting Point, °C	—	199	179
% Nitrogen	7.402	5.664	4.743
% Protein	46.261	35.403	29.643
Digestability Index	0	3	10
Moisture Absorption at 25 % RH at 50% RH at 90% RH	 0.04% 0.07% 0.14%	 0.03% 0.047% 0.15%	 0.00% 0.01% 0.08%

Each person performing laboratory tests is responsible for safety. The analyst should practice good laboratory technique and develop good safety habits to minimize chances for accidents.

3.4 ⚠ Digesdahl Digestion Safety

For safe Digesdahl operation, observe the following precautions:

- Sample size — Never digest a sample which contains over 0.5 g of material which is not water.
- Oils and organic liquids should be considered as solids when determining sample size.
- Acid type — Only use acid specified in Hach step-by-step procedures.
- Acid volume — Never use less than 3 mL.
- Always follow the order of steps indicated.

- If the sample goes to dryness, remove it from the heat immediately and cool to room temperature. **Never add hydrogen peroxide to a dry sample flask; an explosion could occur.** If you are not sure enough sulfuric acid is present in the digestion flask, STOP. Do not add hydrogen peroxide. Begin the digestion again with less sample or more sulfuric acid.
- Be sure to keep the heat shield and the Digesdahl's safety shield in place during use.
- Always perform digestion behind a safety shield (Cat. No. 50040-00) or in a closed fume hood.
- For respiratory protection use a fume hood.
- Always wear safety glasses or goggles– be sure they have side shields.
- Wear protective gloves during digestion procedures. Use tongs or finger cots to transfer hot apparatus.
- **Do not** add alcohol, acetone or other organic solvents to the digestion flask before or after digestion.
- During digestion, use the heat setting and digestion time specified in the instructions. Do not leave the Digesdahl unattended during use.
- When digesting a new substance for the first time, begin with a smaller size and work up to the optimum quantity for digestion. **Do not** permit the flask to boil to dryness.
- Use laboratory coats or aprons to protect skin and clothing from splashes.
- Wear appropriate shoes to protect feet from spills. Open-toed shoes should not be worn.
- Do not use damaged glassware or apparatus. Discard all damaged equipment and replace it.
- Allow the Digesdahl to cool naturally (in ambient air). Cold water may cause hot glassware to shatter.

3.4.1 ⚠ Using Hydrogen Peroxide

DANGER
Hydrogen peroxide is an explosion hazard.

Use these additional specific safety precautions when using hydrogen peroxide in the Digesdahl digestion applications:

- **Do not** mix hydrogen peroxide with any chemical reagents except as specified in the Hach instructions.
- **Do not** add hydrogen peroxide directly to the column on the digestion flask. Always add hydrogen peroxide in a slow and controlled manner; use the capillary funnel.
- Hydrogen peroxide should be added to the organic materials in the flask **only** when sulfuric acid is present.
- Do not use hydrogen peroxide in concentrations greater than 50%.

Hydrogen peroxide (30% or 50%) is a powerful oxidant and should never be stored near flammable materials. Like sulfuric acid, it can cause burns and eye damage. **In case of eye or skin contact, flush eyes and/or skin with water for 15 minutes. Immediately call a physician.**

Hydrogen peroxide is highly corrosive and should be cleaned up with water if spilled on instruments or a counter top. Read and observe all warnings on the reagent labels and Material Safety Data Sheets.

Proper handling and storage procedures involving hydrogen peroxide should always address two major characteristics of the product:

- It is a strong oxidizing agent. The chemical nature of hydrogen peroxide makes it a strong irritant to skin, mucous membranes and particularly to the eyes. It will cause chemical burns at industrial concentrations and may cause spontaneous combustion upon immediate or prolonged contact with combustibles.
- It can decompose, releasing heat and oxygen. Normally this reaction rate is very slow for industrial-grade product, but it will accelerate when contaminated by materials such as dust, metallic ions, or alkali.

Please observe the following precautions for handling and storing of hydrogen peroxide:

- **Do** store in a cool place away from direct sunlight (preferably in a refrigerator).
- **Do** store in the original containers with closures as supplied and keep closed when not in use. (Be sure the containers are vented. Hach hydrogen peroxide bottles are shipped with a special vented cap liner.)
- **Do** wear gloves and safety glasses when handling the material.
- **Do** use silicon carbide boiling chips when digesting liquid samples.
- **Do** wash contaminated skin and body quickly with plenty of water. Remove contaminated clothing and wash well before using again.
- **Do** wash eyes with plenty of water if contaminated and get medical attention quickly.
- **Do** get medical advice without delay if the material is ingested.
- **Do** flush all spills with large amounts of water.
- **Do not** store near heat sources or in contact with combustible or organic materials.
- **Do not** inhale vapors or ingest the material.
- **Do not** allow contact with eyes or skin.
- **Do not** allow contact with decomposition catalysts (metals, dust, alkali, etc.).
- **Do not** use unapproved materials (brass, copper, carbon steel, rubber, etc.) for transfer or storage systems.

Caps on the reagent bottles are made with a special porous liner that allows venting of gas. The venting cap always must be used on the bottle of hydrogen peroxide. As a precaution, the reagent

bottles are shipped in a plastic bag. If there is evidence of leakage during shipment, wear gloves when removing the bottle from the bag and rinse the bottle with water when removed from the bag. Rinse the bag before disposal.

3.4.2 ⚠ Using Sulfuric Acid

Read and observe all warnings on the reagent labels and Material Safety Data Sheets that accompany the sulfuric acid.

Concentrated sulfuric acid used in the digestion process should be handled correctly and with caution. Sulfuric acid is a strong acid and strong oxidizer; it can cause burns if splashed on the skin and permanent damage if eye contact occurs. This caustic action is much more severe if the acid is hot. **In case of eye or skin contact, flush eyes and/or skin with water for 15 minutes. Immediately call a physician.**

Sulfuric acid mist or vapor has been classified by the International Agency for Research on Cancer (IARC) as a possible human carcinogen.

Sulfuric acid is a strong oxidizer. It may ignite or explode on contact with many different chemicals. Follow proper storage regulations.

3.4.3 ⚠ Clean Up of Spills and Leaks

Use extreme caution when cleaning spills and leaks throughout the entire digestion procedure. Your facility may require that only trained individuals wearing appropriate protective equipment (gloves, goggles, face shields and chemical resistant clothing) respond to a spill or leak to ensure the Digesdahl is properly cleaned.

A spill, overflow, or eruption from the Digesdahl apparatus may leave a residue on the equipment or other surfaces. Cleaning the residue must be done cautiously. Do not use alternative cleaning methods or cleaning agents not authorized or endorsed by Hach Company; they may damage the equipment.

While cleaning a spill or leak, follow the safety measures below:

1. DO NOT attempt to clean the apparatus if it is hot; this could cause the glass to shatter. Let the apparatus cool before cleaning it.

2. Unplug the Digesdahl before cleaning.

3. Wear gloves and goggles when handling the glassware. Carefully rinse the glassware several times with water to decontaminate it.

4. Wipe the exterior surfaces of the Digesdahl apparatus (heating mantle, electrical controls, etc.) and other laboratory surfaces several times with a damp or wet cloth or paper towel.

5. Do not rinse or spray the apparatus directly; this could damage the equipment.

6. Discard any paper towels or cloths in an appropriate manner; they may be contaminated from the sample or chemical residues.

3.4.4 Waste Management

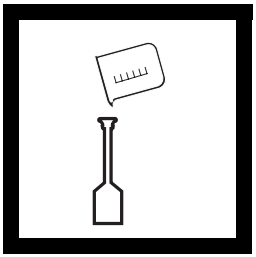
Hazardous waste disposal regulations were promulgated in accordance with the Resource Conservation and Recovery Act (RCRA) and are given in Title 40 Code of Federal Regulations (CFR), parts 260 to 280. Waste must be managed and disposed of in accordance with federal, state and local regulations. Refer to Section VIII of the Hach Material Safety Data Sheet that come with reagents for basic disposal information on Hach Products. The USEPA maintains a hotline number for questions regarding RCRA. It is 1-800-424-9346.

This manual does not contain all the regulatory requirements. Additional state and local laws may apply to waste that you generate. It is each generator's responsibility to know which regulations apply to them and to adhere to these regulations. Check with your environmental compliance staff for specific instructions.

SECTION 3 summarizes basic requirements for safely handling the chemicals used in the Digesdahl digestion. It is a guide only and is not comprehensive for all parties. The information is not intended to provide any express or implied warranties to readers. This information is not intended to form any type of obligation upon Hach Company or its agents.

For further information, please contact the Hach Environmental Safety and Health Department at (515) 232-2533.

Digestion Procedure for Aqueous Liquids



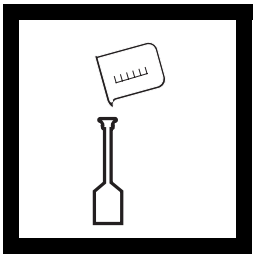
1. Transfer a premeasured amount of sample into a 100-mL Digesdahl digestion flask; ***see Sample and Analysis Volume Tables for Aqueous Liquids following each procedure***. The amount transferred should not contain more than 0.5 g of material which is not water. The maximum volume for water samples is 40 mL. In samples with more than 1% solids present, use the formula below:

Water Sample Volume = 40 ÷ % solids

Note: *Use only Hach Digesdahl flasks. Volumetric flasks with concave bottoms should not be used.*

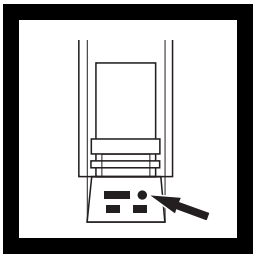
Note: *If solids are 10% of total volume of sample, the maximum volume of liquid sample would be 4 mL.*

Note: *If liquid is too viscous to measure, preweigh the sample into the digestion flask.*



2. Add 3 mL of concentrated sulfuric acid (spec. gravity 1.84) to the volumetric flask and two or more silicon carbide (carborundum) boiling chips for liquid samples.

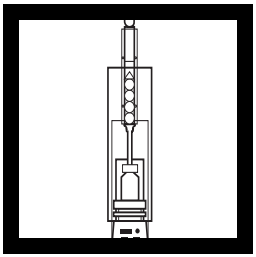
Note: *Pretreat boiling chips by soaking in 1:1 Nitric Acid and rinsing thoroughly with deionized water. Treatment is very important in low-level work. Hach recommends using silicon carbide boiling chips.*



3. Turn the temperature dial to a heat setting of 440 °C (825 °F). When the proper temperature is reached, turn on the water to the aspirator and make sure there is suction to the fractionating column.

Note: *Wait for the proper temperature to be reached before sample is placed on the heater.*

Note: *Always operate the Digesdahl apparatus with a safety shield in place or inside a closed fume hood. Safety glasses are mandatory.*



4. Place the flask weight followed by the fractionating column with funnel on the flask. Place the flask on the heater and heat until the sulfuric acid boils (refluxing sulfuric acid will be visible).

Note: *White acid vapors will usually be present but their presence alone does not indicate that the boiling point of sulfuric acid has been reached.*

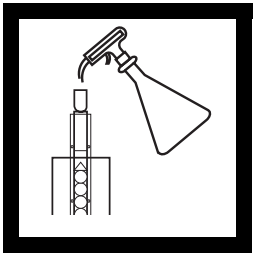
Note: *Aqueous samples require total evaporation of water before vapors are visible.*

Note: *If sample foams up into the neck of the flask, lower temperature to 335 °C (635 °F). Continue heating at a lower temperature until all water is evaporated. Then return to original digestion temperature.*

Note: *If foaming or bumping is not stopped by lowering temperature or volume, then liquid samples that will not clog the capillary funnel may be added to the flask via the capillary funnel, 10 mL at a time. Decrease amount added if foaming persists.*

Digestion Procedure for Aqueous Liquids, continued

5. Boil 4 more minutes. **Do not boil the sample to dryness**. If sulfuric acid is not present in the flask after the 4 minute heating, **do not proceed with step 6!** Discard sample if it evaporates to dryness. Start over and use a larger volume of sulfuric acid in step 2 of this procedure. Or choose a smaller sample amount for digestion.



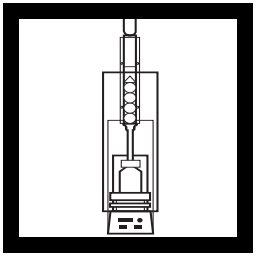
6. Do not proceed if sulfuric acid is not visible in the flask! If sulfuric acid is present, add 10 mL of 50% Hydrogen Peroxide to the charred sample via the funnel on the fractionating column.

Note: *Visually confirm the presence of sulfuric acid in the flask before adding hydrogen peroxide.*

Note: *If the digest does not turn colorless, add 5-mL increments of hydrogen peroxide until the digest becomes clear or does not change color.*

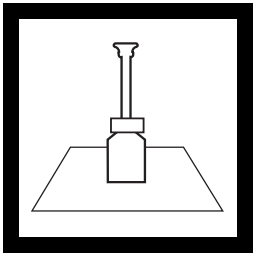
Note: *If sample foams excessively during peroxide addition, remove the Digesdahl digestion flask and fractionating column (use finger cots). Starting with step 1, repeat the digestion adding only 2 mL of hydrogen peroxide. Then follow with 8 mL of hydrogen peroxide.*

Note: *Do not heat to dryness.*



7. After addition of hydrogen peroxide is complete, boil off excess hydrogen peroxide by heating until the acid starts to reflux again and white acid vapors are visible. Do not heat to dryness.

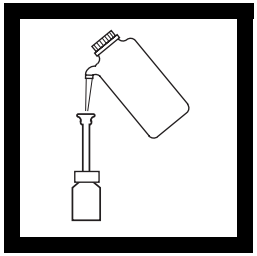
Note: *If the sample goes to dryness, turn off the Digesdahl and air cool to room temperature.* ***Add water to flask before handling.*** *Repeat the digestion from the beginning using a new sample.*



8. Take the hot flask off the heater and allow the flask to cool. Remove the fractionating column from the digestion flask.

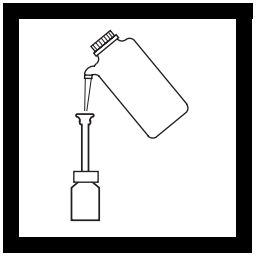
Note: *Use finger cots to remove the digestion flask. Place it on a cooling pad for <u>at least</u> one minute. Then remove the column. Do not add water to the flask until it has cooled.*

Digestion Procedure for Aqueous Liquids, continued

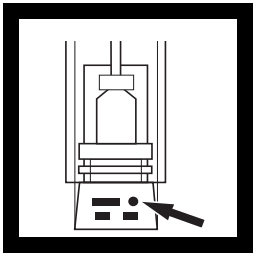


9. Dilute the digest to approximately 70 mL with deionized water.

Note: *Add deionized water slowly at first. Cool the flask if necessary for handling.*



10. If analyzing for aluminum, nickel or iron, continue to step 11. If analyzing for other substances, dilute to the 100-mL mark with deionized water; skip step 11 and go to step 12.



11. Turn the temperature dial to a heat setting of 204 °C (400 °F). Add 150 mL of water to a 400-mL beaker. Place the beaker on the heater. Place the flask in the beaker and boil for 15 minutes. Air cool to room temperature and dilute to the 100-mL mark with deionized water. Invert several times to mix.

Note: *When using a Digesdahl Digestion Apparatus without temperature control dials, reset to a lower setting that gently boils the water.*

12. If the sample has visible turbidity, filter or wait until the turbidity settles, and the upper portion of the sample is clear. Continue with the analysis using the appropriate procedure. For pH adjustment see the instructions following this step.

Filter as follows:

a. Place 47-mm glass filter paper into the filter holder with wrinkled surface upward.

b. Place the filter holder assembly in the filtering flask and wet the filter with deionized water to ensure adhesion to the holder.

c. While applying a vacuum to the filtering flask, transfer the sample to the filtering apparatus.

d. Slowly release the vacuum from the filtering flask and transfer the solution to another container.

pH Adjustment

Metals Method

Note: *If analyzing aliquots smaller than 2% of final volume, pH adjustment is not necessary.*

1. Pipet the appropriate analysis volume into the appropriate mixing graduate cylinder. See Sample and Analysis Volume Tables for Aqueous Liquids in the procedure to determine the analysis volume.

Note: *Some methods require pipetting into a volumetric flask or a regular graduated cylinder.*

2. Dilute to slightly less than the final dilution volume with deionized water.

3. Add one drop of 2,4 Dinitrophenol Indicator Solution.

4. Add one drop of 8 N Potassium Hydroxide (KOH) Standard Solution at a time, swirling between each addition, until the first flash of yellow appears (pH 3). If analyzing for potassium, use 5 N sodium hydroxide instead. Do not use a pH meter if analyzing for potassium or silver.

Note: *Use pH paper to insure the pH is 3. If it is higher than 4, do not readjust with acid; start over with a fresh aliquot.*

5. Add one drop of 1 N KOH. Stopper the cylinder and invert several times to mix. If analyzing for potassium, use 1 N sodium hydroxide instead.

6. Continue to add 1 N KOH in this manner until the first permanent yellow color appears (pH 3.5-4.0).

Note: *High iron content will cause precipitation (brown cloud) which will co-precipitate other metals. Repeat this procedure with a smaller aliquot volume.*

7. Add deionized water to the volume indicated in the colorimetric procedure for the analyte.

8. Continue with the colorimetric procedure for the analyte.

pH Adjustment for TKN

Consult the spectrophotometer or colorimeter procedure to complete the TKN analysis. The following is only a guide to use if a procedure is not available.

1. Pipet an appropriate analysis volume into a graduated mixing cylinder.
2. Add one drop of TKN Indicator.
3. Add 8 N KOH Standard Solution, one drop at a time, swirling between each addition, until the first flash of pale blue appears (pH 3).
4. Add one drop of 1 N KOH. Stopper the cylinder and invert several times to mix.

Note: *View the cylinder from the top against a white background. Compare the cylinder against a second cylinder filled to the same volume with deionized water.*

5. Continue to add 1 N KOH in this manner until the first permanent blue color appears.
6. Add deionized water to the volume indicated in the colorimetric procedure.
7. Continue with the colorimetric procedure.

Digestion Procedure for Aqueous Liquids, continued

REQUIRED REAGENTS

Description	Quantity Required Per Digestion	Unit	Cat. No.
Hydrogen Peroxide, 50%	10 mL	490 mL	21196-49
Potassium Hydroxide Standard Solution, 1 N	varies	50 mL SCDB*	23144-26
Potassium Hydroxide Standard Solution, 8 N	varies	500 mL	282-49
Sulfuric Acid, ACS (conc., specific gravity 1.84)	>3 mL	2.5 liters	979-09
Water, deionized	varies	4 liters	272-56

REQUIRED APPARATUS

Boiling Chips, silicon carbide	varies	500 g	20557-34
Dispenser, pour-out, 10 mL	1	each	22200-38
Flask, flat-bottom, volumetric, w stopper, 100 mL	1	each	23125-42
Pipet, serological, 10 mL	1	each	532-38
Pipet Filler, safety bulb	1	each	14651-00
Safety Glasses	1	each	18421-00
Safety Shield, for Digesdahl®	1	each	50040-00
Select one based on available voltage:			
Digesdahl® Apparatus, 115 V	1	each	23130-20
Digesdahl® Apparatus, 230 V	1	each	23130-21

OPTIONAL REAGENTS

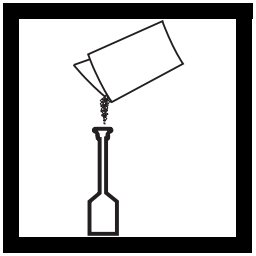
Hydrogen Peroxide, 30%, ACS	200 mL		144-45
Kjeldahl Reduction Reagent (for fluid fertilizers)	40 g		23653-04
2,4-Dinitrophenol Indicator Solution	100 mL MDB		1348-32
Nitric Acid Solution, 1:1	500 mL		2540-49
Sodium Hydroxide, 5 N	50 mL SCDB*		2450-26
Sodium Hydroxide, 1 N	900 mL		1045-53
TKN Indicator Solution	50 mL SCDB		22519-26

* Contact Hach for larger sizes.

OPTIONAL APPARATUS

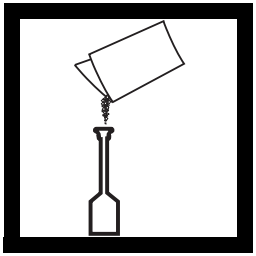
Description	Unit	Cat. No.
Balance, Precision, 115 V	each	26104-00
Balance, Precision, 220 V	each	26104-02
Beaker, 400 mL	each	500-48
Beaker, Berzelius, 200 mL	12/pkg	22761-75
Bottle, Wash, 1 L	each	620-16
Bulb, dropper, 2 mL	12/pkg	21189-00
Cylinder, graduated, 50 mL	each	508-41
Dispenser, Digital, 1.0-5.0 mL (for sulfuric acid	each	25631-37
Dispenser, Digital, 5-59 mL (for sulfuric acid)	each	25631-41
Filter Discs, glass, 47mm	100/pkg	2530-00
Filter Holder, glass, 47 mm	each	2340-00
Flask, filter, 500 mL	each	546-49
Oven, laboratory, 120 V	each	14289-00
Paper, weighing, 76 x 76 mm	500/pkg	14738-00
pH Paper, pH 1-11	5 rolls/pkg	391-33
pH Meter, *sens**ion***™***1***, portable	each	51700-10
Pipet, Pasteur, disposable, 229 mm	200/pkg	21234-01
Safety Goggles	each	18421-00
Spatula, stainless, 10 cm	each	561-64
Spoon, measuring, 0.05 g	each	492-00
Stir Bar, Teflon-coated, 25.4 x 7.9 mm	each	20953-51
Stir Plate, magnetic, Thermolyne, 120V, 50/60 Hz	each	23444-00
Stir Plate, magnetic, Thermolyne, 240V, 50/60 Hz	each	23444-02
Stopper, hollow, size #5	6/pkg	14480-05
Syringe, 5 cc, plastic	100/pkg	23433-33
Watch Glass, 65 mm	12/pkg	578-97

Digestion Procedure for Solids



1. Transfer 0.50 g or less of sample into a 100-mL Digesdahl digestion flask; see Sample and Analysis Volume Table for Solids following each procedure.

Note: *Be sure you have a homogenous sample. Solid samples should be finely ground or chopped and mixed well.*

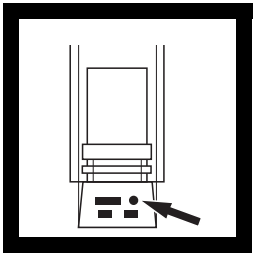


2. Add 4 mL concentrated sulfuric acid (spec. gravity 1.84) to the digestion flask.

Note: *Use only Hach Digesdahl digestion flasks. Volumetric flasks with concave bottoms should not be used.*

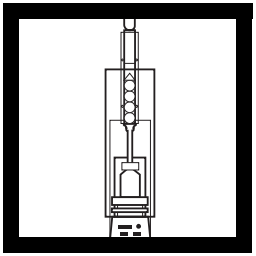
Note: *Add at least 4 mL of acid to solid samples. See Table 3 on page 24 for recommendations.*

Note: *Safety glasses and a safety shield placed between the operator and the Digesdahl are required.*



3. Turn the temperature dial to a heat setting of 440 °C (825 °F). When the proper temperature is reached, turn on the water to the aspirator and make sure there is suction to the fractionating column.

Note: *Wait for the proper temperature to be reached before sample is placed on the heater.*



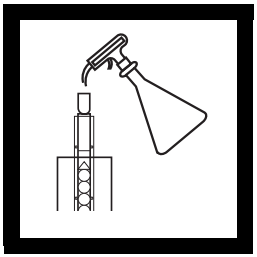
4. Place the flask weight followed by the fractionating column with funnel on the flask. Place the flask on the heater and boil 4 minutes. **Do not boil to dryness! If sulfuric acid is not present in the flask after the boiling period, do not proceed to step 5!** Discard the sample and use more sulfuric acid for the digestion procedure in step 2. Or choose a smaller amount from the Sample and Analysis Volume Tables for Solids.

Note: *If sample foams up into the neck of the flask, lower temperature to 335 ˚C (635 ˚F). Continue heating at lower temperature until all water is evaporated. Then return to original digestion temperature.*

Note: *White acid vapors accompanied with a reflux line indicate that the sulfuric acid is boiling.*

Note: *Some organic samples may need more than 4 minutes for complete digestion.*

Digestion Procedure for Solids, continued



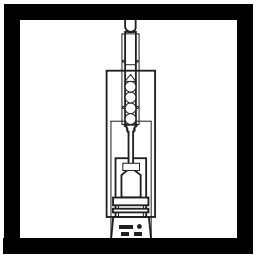
5. Do not proceed if sulfuric acid is not visible in the flask. Add 10 mL of 50% hydrogen peroxide to the charred sample via the funnel on the fractionating column.

Note: *Do not heat to dryness.*

Note: *Visually confirm the presence of sulfuric acid in the flask before adding hydrogen peroxide.*

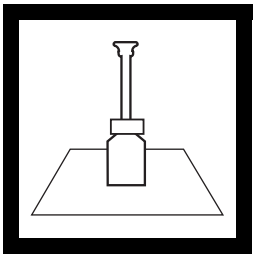
Note: *If the digest does not turn colorless, add 5 mL increments of peroxide until the digest becomes clear or does not change color.*

Note: *If sample foams excessively during hydrogen peroxide addition, stop the peroxide flow and remove the digestion flask and fractionating column (use finger cots). Cool for 30 seconds and return apparatus to the heating block. Start peroxide addition with 2 mL, then follow with the remaining peroxide.*



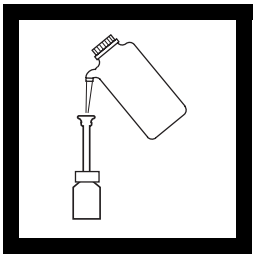
6. After addition of hydrogen peroxide is complete, boil off excess hydrogen peroxide by heating until the acid starts to reflux again and white acid vapors are visible. **Do not heat to dryness.**

Note: *If the sample goes to dryness, turn off the Digesdahl and air cool to room temperature.* ***Add water to flask before handling.*** *Repeat the digestion from the beginning using a new sample.*



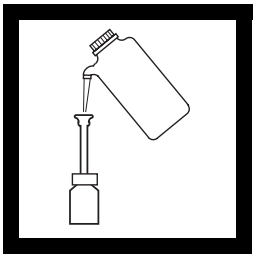
7. Take the hot flask off the heater and allow the flask to air cool. Remove the fractionating column from the digestion flask.

Note: *Use finger cots to remove the digestion flask. Place it on a cooling pad for at least one minute. Then remove the column. Do not add water to the flask until it has cooled.*

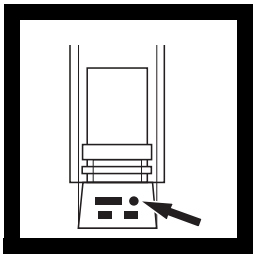


8. Dilute the digest to approximately 70 mL with deionized water.

Note: *Add deionized water slowly at first. Cool the flask if necessary for handling.*



9. If analyzing for aluminum, nickel or iron, continue to step 10. If analyzing for other substances, dilute to the 100-mL mark with deionized water; skip step 10 and go to step 11.



10. Turn the temperature dial to a heat setting of 204 °C (400 °F). Add 150 mL of water to a 400-mL beaker. Place the beaker on the heater. Place the flask in the beaker and boil for 15 minutes. Air cool to room temperature and dilute to the mark with deionized water. Invert several times to mix.

Note: *When using a Digesdahl Digestion Apparatus system without temperature control dials, reset to a lower setting that gently boils the water.*

11. If the sample has visible turbidity, filter or wait until the turbidity settles, and the upper portion of the sample is clear.

Filter as follows:

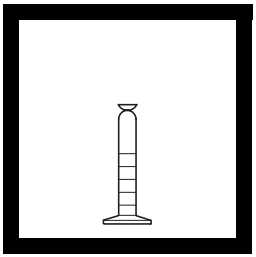
Place 47-mm glass filter paper into the filter holder with wrinkled surface upward.

Place the filter holder assembly in the filtering flask and wet the filter with deionized water to ensure adhesion to the holder.

While applying a vacuum to the filtering flask, transfer the sample to the filtering apparatus.

Slowly release the vacuum from the filtering flask and transfer to another container.

Note: *If small aliquots (≤1.0 mL) are analyzed, filtration is not needed.*



12. Continue with the analysis using the appropriate procedure. For pH adjustment see the instructions following his step.

pH Adjustment

Metals Method

Note: *If analyzing aliquots smaller than 2% of the final diluted volume, pH adjustment is not necessary.*

1. Pipet the appropriate analysis volume into the appropriate mixing graduate cylinder. See Sample and Analysis Volume Tables for Solids following the specific digestion to determine the analysis volume.

Note: *Some methods require pipetting into a volumetric flask or a regular graduated cylinder.*

2. Dilute to slightly less than the final dilution volume with deionized water.

3. Add one drop of 2,4 Dinitrophenol Indicator Solution.

4. Add one drop of 8 N Potassium Hydroxide (KOH) Standard Solution at a time, swirling between each addition, until the first flash of yellow appears (pH 3). If analyzing for potassium, use 5 N sodium hydroxide instead. Do not use a pH meter if analyzing for potassium or silver.

Note: *Use pH paper to insure the pH is 3. If it is higher than 4, do not readjust with acid; start over with a fresh aliquot.*

5. Add one drop of 1 N KOH. Stopper the cylinder and invert several times to mix. If analyzing for potassium, use 1 N sodium hydroxide instead.

6. Continue adding 1 N KOH in this manner until the first permanent yellow color appears (pH 3.5-4.0).

Note: *High iron content will cause precipitation (brown cloud) which will co-precipitate other metals. Repeat this procedure with a smaller aliquot volume.*

7. Add deionized water to the volume indicated in the colorimetric procedure for the analyte.

8. Continue with the colorimetric procedure for the analyte.

pH Adjustment For TKN

Consult the spectrophotometer or colorimeter procedure to complete the TKN analysis. The following is only a guide to use if a procedure is not available.

1. Pipet an appropriate analysis volume into a graduated mixing cylinder.
2. Add one drop of TKN Indicator.
3. Add 8 N KOH Standard Solution, one drop at a time, swirling between each addition, until the first flash of pale blue appears (pH 3).
4. Add one drop of 1 N KOH. Stopper the cylinder and invert several times to mix.
5. View the cylinder from the top against a white background. Compare the cylinder against a second cylinder filled to the same volume with deionized water.
6. Continue to add 1 N KOH in this manner until the first permanent blue color appears.
7. Add deionized water to the volume indicated in the colorimetric procedure.
8. Continue with the colorimetric procedure.

Digestion Procedure for Solids, continued

REQUIRED REAGENTS

Description	Quantity Required Per Digestion	Unit	Cat. No.
Hydrogen Peroxide, 50%	10 mL	490 mL	21196-49
Potassium Hydroxide Standard Solution, 1 N	varies	50 mL SCDB*	23144-26
Potassium Hydroxide Standard Solution, 8 N	varies	500 mL	282-49
Sulfuric Acid, ACS (conc., specific gravity 1.84)	>3 mL	2.5 liters	979-09
Water, deionized	varies	4 liters	272-56

REQUIRED APPARATUS

Boiling Chips, silicon carbide	varies	500 g	20557-34
Dispenser, pour-out, 10 mL	1	each	22200-38
Flask, flat-bottom, volumetric, w/ stopper, 100 mL	1	each	23125-42
Pipet, serological, 10 mL	1	each	532-38
Pipet Filler, safety bulb	1	each	14651-00
Safety Glasses	1	each	18421-00
Safety Shield, for Digesdahl®	1	each	50040-00
Select one based on available voltage:			
Digesdahl® Apparatus, 115 Vac	1	each	23130-20
Digesdahl® Apparatus, 230 Vac	1	each	23130-21

OPTIONAL REAGENTS

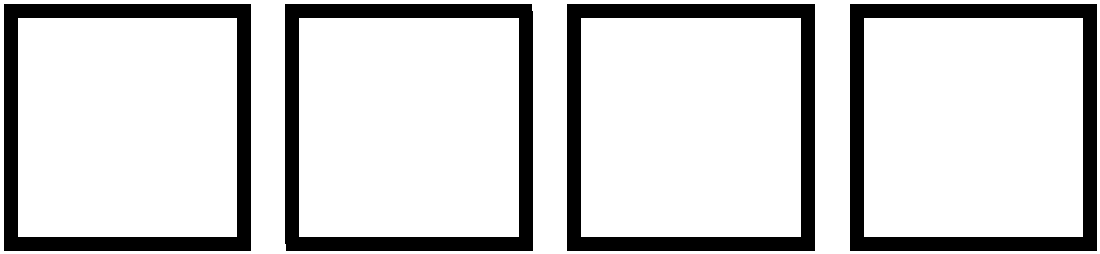
Hydrogen Peroxide, 30%, ACS	200 mL		144-45
Kjeldahl Reduction Reagent (for fluid fertilizers)	40 g		23653-04
2,4-Dinitrophenol Indicator Solution	100 mL MDB		1348-32
Nitric Acid Solution, 1:1	500 mL		2540-49
Sodium Hydroxide, 5 N	50 mL SCDB*		2450-26
Sodium Hydroxide, 1 N	900 mL		1045-53
TKN Indicator Solution	50 mL SCDB		22519-26

* Contact Hach for larger sizes.

OPTIONAL APPARATUS

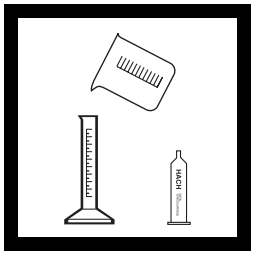
Description	Unit	Cat. No.
Balance, Precision, 115 V	each	26104-00
Balance, Precision, 220 V	each	26104-02
Beaker, 400 mL	each	500-48
Beaker, Berzelius, 200 mL	12/pkg	22761-75
Bottle, Wash, 1 L	each	620-16
Bulb, dropper, 2 mL	12/pkg	21189-00
Cylinder, graduated, 50 mL	each	508-41
Dispenser, Digital, 0.5-5.9 mL (for sulfuric acid, meat)	each	25631-37
Dispenser, Digital, 5-59 mL (for sulfuric acid, meat)	each	25631-41
Filter Discs, glass, 47mm	100/pkg	2530-00
Filter Holder, glass, 47 mm	each	2340-00
Flask, filter, 500 mL	each	546-49
Oven, laboratory, 120 V	each	14289-00
Paper, weighing, 76 x 76 mm	500/pkg	14738-00
pH Paper, pH 1-11	5 rolls/pkg	391-33
pH Meter, *sens**ion***™*1*, portable	each	51700-10
Pipet, Pasteur, disposable, 229 mm	200/pkg	21234-01
Safety Goggles	each	18421-00
Spatula, stainless, 10 cm	each	561-64
Spoon, measuring, 0.05 g	each	492-00
Stir Bar, Teflon-coated, 25.4 x 7.9 mm	each	20953-51
Stir Plate, magnetic, Thermolyne, 120V, 50/60 Hz	each	23444-00
Stir Plate, magnetic, Thermolyne, 240V, 50/60 Hz	each	23444-02
Stopper, hollow, size #5	6/pkg	14480-05
Syringe, 5 cc, plastic	100/pkg	23433-33
Watch Glass, 65 mm	12/pkg	578-97

SECTION 4 ANALYSIS METHODS



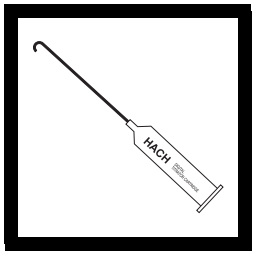
ALKALINITY, TOTAL & PHENOLPHTHALEIN

Using Sulfuric Acid and the Digital Titrator Range: 10-4000 mg/L as $CaCO_3$

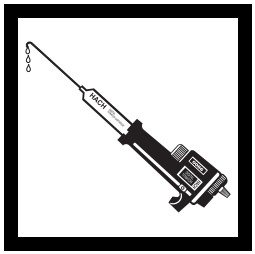


1. Select the sample volume and Sulfuric Acid (H_2SO_4) Titration Cartridge corresponding to the expected alkalinity concentration as mg/L calcium carbonate ($CaCO_3$) from *Table 1*.

Note: *See Sampling and Storage following these steps.*

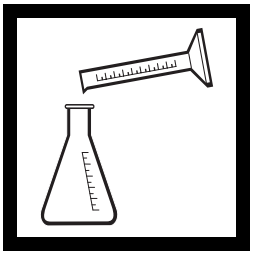


2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.



3. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

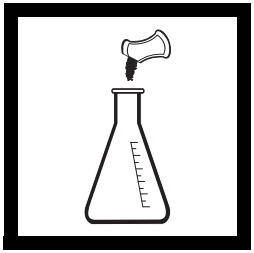
Note: *For added convenience use the TitraStir stirring apparatus.*



4. Use a graduated cylinder or pipet to measure the sample volume from *Table 1*. Transfer the sample into a clean 250-mL Erlenmeyer flask. Dilute to about the 100-mL mark with deionized water, if necessary.

Table 1

Range (mg/L)	Sample Volume (mL)	Titration Cartridge	Catalog Number	Digit Multiplier
10-40	100	0.1600	14388-01	0.1
40-160	25	0.1600	14388-01	0.4
100-400	100	1.600	14389-01	1.0
200-800	50	1.600	14389-01	2.0
500-2000	20	1.600	14389-01	5.0
1000-4000	10	1.600	14389-01	10.0



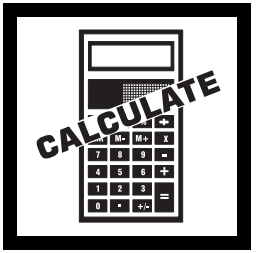
5. Add the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix.

Note: *A solution of one pH 8.3 Buffer Powder Pillow and one Phenolphthalein Powder Pillow in 50 mL of deionized water is recommended as a comparison for determining the proper end-point color.*

Note: *Four drops of Phenolphthalein Indicator Solution may be substituted for the Phenolphthalein Indicator Powder Pillow.*

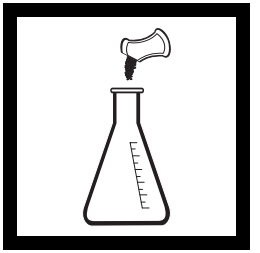
6. If the solution turns pink, titrate to a colorless end point. Place the delivery tube tip into the solution and swirl the flask while titrating with sulfuric acid. Record the number of digits required.

Note: *If the solution is colorless before titrating with Sulfuric acid, the Phenolphthalein (P) alkalinity is zero; proceed with step 8.*



7. Calculate:

Digits Required x
Digit Multiplier =
mg/L $CaCO_3$ P Alkalinity



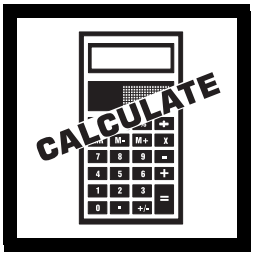
8. Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the flask and swirl to mix.

Note: *Four drops of Methyl Purple Indicator Solution may be substituted for the Bromcresol Green-Methyl Red Indicator Powder Pillow. Titrate from green to a gray end point (pH 5.1).*

Note: *Four drops of Bromcresol Green-Methyl Red Indicator Solution may be substituted for the Bromcresol Green-Methyl Red Indicator Powder Pillow.*

9. Continue the titration with sulfuric acid to a light greenish blue-gray (pH 5.1), a light violet-gray (pH 4.8), or a light pink (pH 4.5) color, as required by the sample composition; see *Table 3.* Record the number of digits required.

Note: *A solution of one Bromcresol Green-Methyl Red Powder Pillow and one pillow of the appropriate pH buffer in 50 mL of deionized water is recommended as a comparison for judging the proper end-point color. If the pH 3.7 end point is used, use a Bromphenol Blue Powder Pillow instead of a Bromcresol Green-Methyl Red and titrate to a green end point.*



10. Calculate:

Total Digits Required x Digit Multiplier = mg/L as $CaCO_3$ Total (T or M) Alkalinity

Note: *Carbonate, bicarbonate and hydroxide concentrations may be expressed individually using the relationships shown in Table 3 on page 54.*

Note: *meq/L Alkalinity = mg/L as $CaCO_3$ ÷ 50.*

Table 2

Sample Composition	End Point
Alkalinity about 30 mg/L	pH 5.1
Alkalinity about 150 mg/L	pH 4.8
Alkalinity about 300 mg/L	pH 4.5
Silicates or Phosphonates present	pH 4.5
Industrial waste or complex system	pH 3.7

Sampling and Storage

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation or prolonged exposure to air. Samples should be analyzed as soon as possible after collection but can be stored at least 24 hours by cooling to 4 °C (39 °F) or below. Warm to room temperature before analyzing.

Alkalinity Relationship Table

Total alkalinity primarily includes hydroxide, carbonate and bicarbonate alkalinities. The concentration of these alkalinities in a sample may be determined when the phenolphthalein and total alkalinities are known (see *Table 3*).

Table 3 Alkalinity Relationship

Row	Result of Titration	Hydroxide Alkalinity is equal to:	Carbonate Alkalinity is equal to:	Bicarbonate Alkalinity is equal to:
1	Phenolphthalein Alkalinity = 0	0	0	Total Alkalinity
2	Phenolphthalein Alkalinity equal to Total Alkalinity	Total Alkalinity	0	0
3	Phenolphthalein Alkalinity less than one half of Total Alkalinity	0	2 times the Phenolphthalein Alkalinity	Total Alkalinity minus two times Phenolphthalein Alkalinity
4	Phenolphthalein Alkalinity equal to one half of Total Alkalinity	0	Total Alkalinity	0
5	Phenolphthalein Alkalinity greater than one half of Total Alkalinity	2 times the Phenolphthalein minus Total Alkalinity	2 times the difference between Total and Phenolphthalein Alkalinity	0

To use the table follow these steps:

a. Does the phenolphthalein alkalinity equal zero? If yes, use Row 1.

b. Does the phenolphthalein alkalinity equal total alkalinity? If yes, use Row 2.

c. Multiply the phenolphthalein alkalinity by 2.

d. Select Row 3, 4, or 5 based on comparing the result of step c with the total alkalinity.

e. Perform the required calculations in the appropriate row, if any.

f. Check your results. The sum of the three alkalinity types will equal the total alkalinity.

For example:
A sample has 170 mg/L as $CaCO_3$ phenolphthalein alkalinity and 250 mg/L as $CaCO_3$ total alkalinity. What is the concentration of hydroxide, carbonate and bicarbonate alkalinities?

The phenolphthalein alkalinity does not equal 0 (it is 170 mg/L), see step a.

The phenolphthalein alkalinity does not equal total alkalinity (170 mg/L vs. 250 mg/L), see step b.

The phenolphthalein alkalinity multiplied by 2 = 340 mg/L, see step c.

Because 340 mg/L is greater than 250 mg/L, select Row 5, see step d.

The hydroxide alkalinity is equal to: (See step e)

340 – 250 = 90 mg/L hydroxide alkalinity

The carbonate alkalinity is equal to:

$250 - 170 = 80$

$80 \times 2 = 160$ mg/L carbonate alkalinity

The bicarbonate alkalinity equals 0 mg/L.

Check: (See step f)

90 mg/L hydroxide alkalinity + 160 mg/L carbonate alkalinity + 0 mg/L bicarbonate alkalinity = 250 mg/L

The above answer is correct; the sum of each type equals the total alkalinity.

Accuracy Check

Standard Additions Method

This accuracy check should be performed when interferences are suspected or to verify analytical technique.

a. Snap the neck off an Alkalinity Voluette Ampule Standard, 0.500 N.

b. Use a TenSette Pipet to add 0.1 mL of standard to the sample titrated in steps 6 or 9. Resume titration back to the same end point. Record the number of digits needed.

c. Repeat, using two more additions of 0.1 mL. Titrate to the end point after each addition.

d. Each 0.1 mL addition of standard should require 25 additional digits of 1.600 N titrant or 250 digits of 0.1600 N titrant. If these uniform increases do not occur, an interference is likely.

Interferences

- Highly colored or turbid samples may mask the color change at the end point. Use a pH meter for these samples.
- Chlorine may interfere with the indicators. Add one drop of 0.1 N Sodium Thiosulfate to eliminate this interference.

Summary of Method

The sample is titrated with sulfuric acid to a colorimetric end point corresponding to a specific pH. Phenolphthalein alkalinity is determined by titration to a pH of 8.3, as evidenced by the color change of phenolphthalein indicator, and indicates the total hydroxide and one half the carbonate present. M (methyl orange) or T (total) alkalinity is determined by titration to a pH between 3.7 and 5.1, and includes all carbonate, bicarbonate and hydroxide.

REQUIRED REAGENTS (varies with sample characteristics)

Description	Cat. No
Alkalinity Reagent Set (about 100 tests)	22719-00
Includes: (1) 943-99, (1) 942-99, (1) 14389-01, (1) 14388-01	

Description	Quantity Required Per Test	Unit	Cat. No
Bromcresol Green-Methyl Red Powder Pillows	1	100/pkg	943-99
Phenolphthalein Powder Pillows	1	100/pkg	942-99
Sulfuric Acid Titration Cartridge, 1.600 N	varies	each	14389-01
Sulfuric Acid Titration Cartridge, 0.1600 N	varies	each	14388-01
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Digital Titrator	1	each	16900-01
Flask, Erlenmeyer, 250 mL	1	each	505-46
Select one or more based on sample concentration:			
Cylinder, graduated, 10 mL	1	each	508-38
Cylinder, graduated, 25 mL	1	each	508-40
Cylinder, graduated, 50 mL	1	each	508-41
Cylinder, graduated, 100 mL	1	each	508-42

OPTIONAL REAGENTS

Description	Unit	Cat. No
Alkalinity Standard Solution, Voluette™ Ampule, 0.500 N Na_2CO_3, 10 mL	16/pkg	14278-10
Bromcresol Green-Methyl Red Indicator Solution	100 mL MDB	23292-32
Bromphenol Blue Indicator Solution	100 mL MDB	14552-32
Bromphenol Blue Powder Pillows	100/pkg	14550-99
Buffer Powder Pillows, pH 3.7	25/pkg	14551-68
Buffer Powder Pillows, pH 4.5	25/pkg	895-68
Buffer Powder Pillows, pH 4.8	25/pkg	896-68
Buffer Powder Pillows, pH 5.1	25/pkg	897-68
Buffer Powder Pillows, pH 8.3	25/pkg	898-68
Methyl Purple Indicator Solution	100 mL MDB	21934-32
Phenolphthalein Indicator Solution, 5 g/L	100 mL MDB*	162-32
Sodium Thiosulfate Standard Solution, 0.1 N	100 mL MDB	323-32

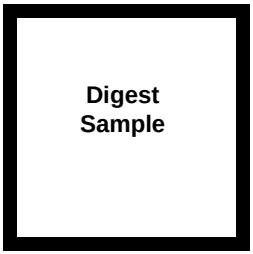
OPTIONAL APPARATUS

Bottle, wash, poly, 500 mL	each	620-11
Clamp, 2-prong extension, 38 mm	each	21145-00
Clamp Holder	each	326-00
Demineralizer Assembly, 473 mL	each	21846-00
Delivery Tubes, with 180° hook	5/pkg	17205-00
Delivery Tubes, 90° with hook for TitraStir®	5/pkg	41578-00
pH Meter, *sens**ion**™1*, portable (with probe)	each	51700-10
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet tips for 19700-01 TenSette® pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 10 mL	each	14515-38
Pipet, volumetric, Class A, 20 mL	each	14515-20
Pipet, volumetric, Class A, 25 mL	each	14515-40
Pipet, volumetric, Class A, 50 mL	each	14515-41
Pipet, volumetric, Class A, 100 mL	each	14515-42
Pipet Filler, safety bulb	each	14651-00
Support ring stand	each	563-00
TitraStir® Mixer/Stand Assembly, 115 Vac	each	19400-00
TitraStir® Mixer/Stand, Assembly, 230 Vac	each	19400-10

* Contact Hach for larger sizes.

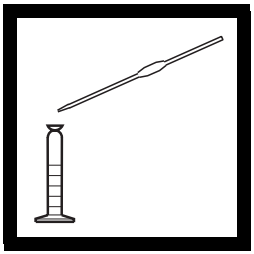
ALUMINUM, TOTAL

Aluminon Method
Range: Liquids- 0.1– 8000 mg/L; Solids- 10–80000 mg/kg



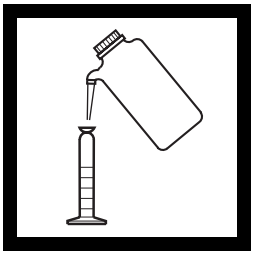
1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *SECTION 3*.

Note: *For best results, analyze a reagent blank for each lot of reagents. Subtract the blank value from the displayed sample value.*



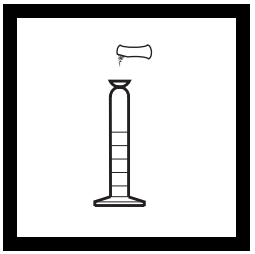
2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 50-mL mixing cylinder. If the aliquot is more than 0.5 mL, adjust the pH according to the instructions following the digestion method.

Note: *Sample temperature must be 20–25 °C (68–77 °F) for accurate results.*

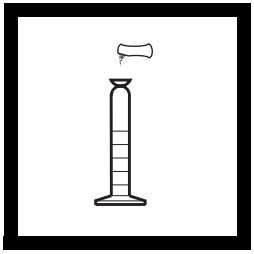


3. Dilute to the 50-mL mark with deionized water.

Note: *Presoak all glassware with warm 1:1 hydrochloric acid. Rinse well with deionized water.*



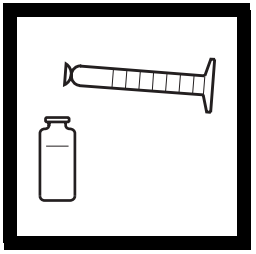
4. Add the contents of one Ascorbic Acid Powder Pillow. Stopper. Invert several times to dissolve powder.



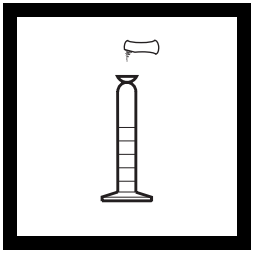
5. Add the contents of one AluVer 3 Aluminum Reagent Powder Pillow. Stopper. Invert repeatedly for one minute to dissolve.

Note: *A red-orange color develops if aluminum is present.*

Note: *Inconsistent results will be obtained if any powder is undissolved.*

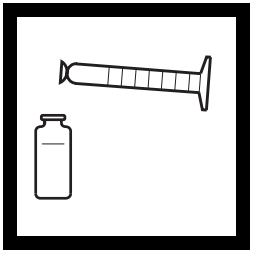


6. Pour 25 mL of mixture into a 25-mL sample cell (the prepared sample).



7. Add contents of one Bleaching 3 Reagent Powder Pillow to the remaining 25 mL in the mixing cylinder. Stopper. Vigorously shake for 30 seconds.

Note: *This solution should turn a light to medium orange upon bleaching. It will not become colorless.*



8. Pour the remaining 25 mL of mixture in the cylinder into a second 25-mL sample cell (the blank).

Note: *The Flow-Thru cell cannot be used.*

9. Wait 15 minutes for the color to develop before completing *step 10*.

10. Zero the instrument with the blank, using the settings below.

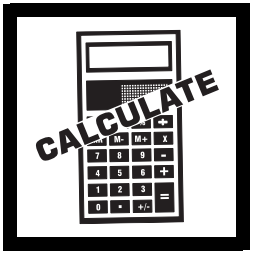
DR/800s
Program No. 1

DR/2010
Program No. 10
522 nm

DR/4000
Program No.1000
522 nm

11. Place the sample in the cell holder. Read the mg/L aluminum.

Note: *For solid samples, express results as mg/kg, not mg/L.*



12. Use the equation below the aluminum Sample and Analysis Volume Tables to calculate the true aluminum concentration.

Note: *Clean the glassware with soap and a brush immediately following the test. If using the same glassware for this test, rinse with HCl and deionized water to remove traces of bleaching reagent.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Al Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute to
0.1-5	40.0	20.0	50.0 mL
0.5-20	20.0	10.0	50.0 mL
2.0-80	10.0	5.00	50.0 mL
20-800	5.00	1.00	50.0 mL
200-8000	1.00	0.50	50.0 mL

Solids

Expected Al Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
10-400	0.500	20.0	50 mL
25-1000	0.400	10.0	50 mL
70-2600	0.300	5.00	50 mL
500-20000	0.200	1.00	50 mL
2000-80000	0.100	0.50	50 mL

Calculation For Final Concentration:

$$\frac{A \times 5000}{B \times C} = \text{mg/L or mg/kg Total aluminum}$$

A = mg/L reading from instrument

B = mL or g sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in a clean glass or plastic container. Preserve the sample by adjusting the pH to 2 or less with nitric acid (about 1.5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 3.5–4.5 with 5.0 N Sodium Hydroxide. Correct results for volume additions.

Accuracy Check

Standard Additions Method

a. Perform the aluminum method and note the analysis volume used and mg/L Al of the sample in *step 11*.

b. Pipet the same analysis volume into three 50-mL graduated mixing cylinders.

c. Snap the neck off an Aluminum Voluette Ampule Standard Solution, 50 mg/L as Al.

d. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to the 50-mL samples. Dilute to 50 mL, if necessary. Mix each thoroughly.

e. Analyze each sample as described above, beginning with *step 4*. The aluminum concentration should increase 0.1 mg/L for each 0.1 mL of standard added.

f. If these increases do not occur, an interference is likely.

Standard Solution Method

a. Prepare a 0.4-mg/L aluminum standard solution by pipetting 1.00 mL of Aluminum Standard Solution, 100 mg/L as Al^{3+}, into a 250-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution immediately before use.

b. Pour 50 mL of the 0.4-mg/L standard into a 500-mL graduated mixing cylinder. Perform *steps 4-11* of the aluminum procedure as described above. The mg/L Al reading in *step 11* should be 0.4 mg/L Al.

Interferences

The following do not interfere up to the indicated concentrations.

Alkalinity	1000 mg/L as $CaCO_3$*
Iron	20 mg/L
Phosphate	50 mg/L

* Check alkalinity with Hach Test Strip 27448-50 and iron with Hach Test Strip 27453-25. Do a 1:10 dilution before testing.

Acidity interferes at greater than 300 mg/L as $CaCO_3$. Treat samples with greater than 300 mg/L acidity as $CaCO_3$ as follows:

a. Add one drop of m-Nitrophenol Indicator Solution to the sample taken in *step 2*.

b. Add one drop of 5.0 N Sodium Hydroxide Standard Solution. Stopper the cylinder. Invert to mix. Repeat as often as necessary until the color changes from colorless to yellow.

c. Add one drop of 5.25 N Sulfuric Acid Standard Solution to change the solution from yellow back to colorless. Continue with the test.

Calcium does not interfere.

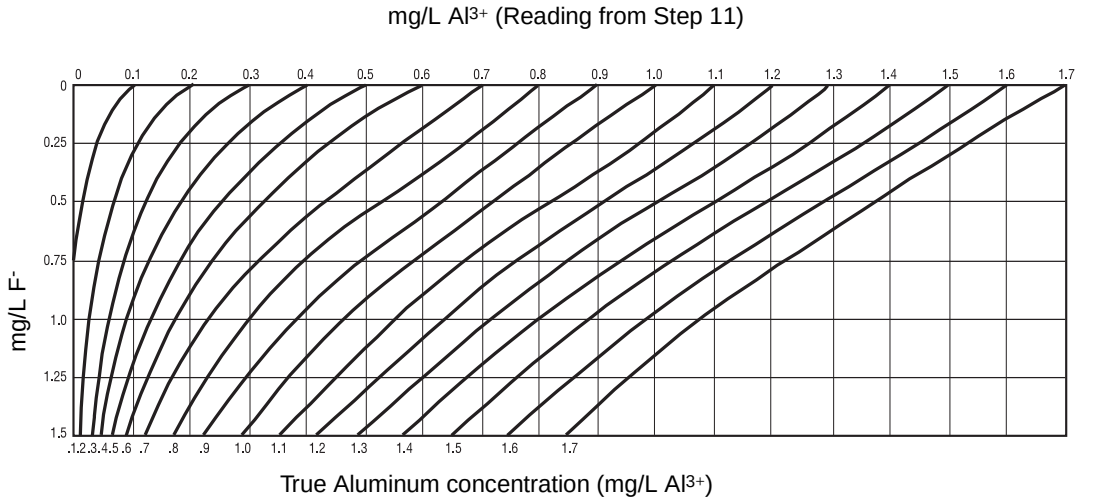
Fluoride interferes at all levels by complexing with aluminum. The actual aluminum concentration can be determined using the Fluoride Interference Graph when the fluoride concentration is known. To use the fluoride interference graph:

1. Select the vertical grid line along the top of the graph that represents the aluminum reading obtained in step 11.

2. Locate the point of the vertical line (*step 11* reading) where it intersects with the horizontal grid line that indicates how much fluoride is present in the sample.

3. Extrapolate the true aluminum concentration by following the curved lines on either side of the intersect point down to the true aluminum concentration. Example: the aluminum test result was 0.7 mg/L Al^{3+} and the fluoride in the sample was 1.0 mg/L F^-. The point where the 0.7 mg/L aluminum grid line intersects with the 1.0 mg/L F^- grid line falls between the 1.2 and 1.3 mg/L Al curves. In this case, the true aluminum content would be 1.27 mg/L.

Figure 3 Fluoride Interference Graph



Summary of Method

Aluminon indicator combines with aluminum in the sample to form a red-orange color. The intensity of color is proportional to the aluminum concentration. Ascorbic acid is added to remove iron interference. The AluVer 3 Aluminum Reagent, packaged in powder form shows exceptional stability and is applicable for fresh water samples.

REQUIRED REAGENTS

Description	Cat. No.
Aluminum Reagent Set (100 Tests)	22420-00
Includes: (4) 14290-46, (1) 14577-99, (1) 14294-49	

Description	Quantity Required Per Test	Unit	Cat. No.
AluVer® 3 Aluminum Reagent Powder Pillow	1 pillow	100/pkg	14290-99
Ascorbic Acid Powder Pillow	1 pillow	100/pkg	14577-99
Bleaching 3 Reagent Powder Pillow	1 pillow	100/pkg	14294-49
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Cylinder, graduated, mixing, 50 mL	1	each	1896-41

Select one or more based an sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

OPTIONAL REAGENTS

Aluminum Standard Solution, 100 mg/L	100 mL	14174-42
Aluminum Standard Solution, Voluette™ ampule, 50 mg/L as Al, 10 mL	16/pkg	14792-10
m-Nitrophenol Indicator Solution, 10 g/L	100 mL	2476-32
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32
Sodium Hydroxide Standard Solution, 5.0 N	50 mL MDB	2450-26
Sulfuric Acid Standard Solution, 5.25 N	100 mL	2449-32
Water Quality Test Strips, total alkalinity, 0-240 ppm	50/pkg	27448-50
Water Quality Test Strips, total iron, 0-5 ppm	25/pkg	27453-25

OPTIONAL APPARATUS

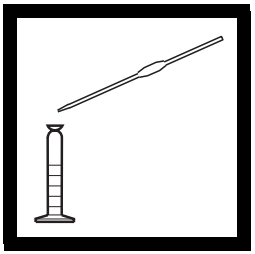
Description	Unit	Cat. No.
Ampule Breaker Kit	each	21968-00
Brush, for glassware	each	690-00
Flask, volumetric, 100 mL	each	547-42
Flask, volumetric, 250 mL	each	547-46
Fluoride Combination Electrode	each	50265-00
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
pH/ISE Meter, *sens**ion***™***2**, portable	each	51725-10
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, TenSette®, 1.0 to 10.0 mL	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	50/pkg	21997-96
Thermometer, -10 to 110 °C	each	1877-01

ALUMINUM, TOTAL

Eriochrome Cyanide R (ECR) Method*
Range: Liquids- 0.05–2200 mg/L; Solids- 4–22000 mg/kg

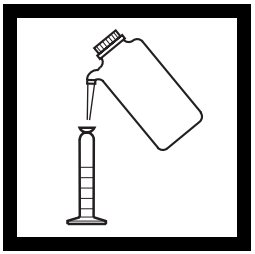
1. Select the sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

Note: *For best results, analyze a reagent blank for each lot of reagents. Subtract the blank value from the displayed sample value.*



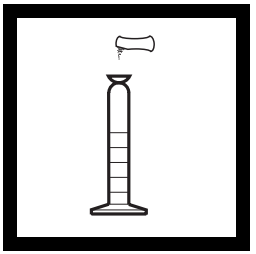
2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 25-mL mixing cylinder. If the aliquot is more than 0.2 mL, adjust the pH according to the instruction following the digestion method.

Note: *Rinse cylinder with 1:1 hydrochloric acid and deionized water before use to avoid errors.*



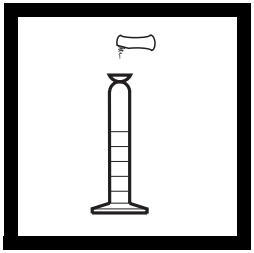
3. Dilute to the 20-mL mark with deionized water.

Note: *The sample temperature must be 20-25 °C (68-77 °F).*



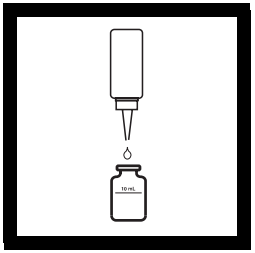
4. Add the contents of one ECR Reagent Powder Pillow. Stopper. Invert several times to dissolve powder, then wait 30 seconds.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*, 3500-Al D.

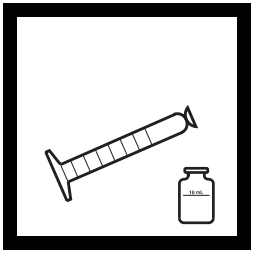


5. Add the contents of one Hexamethylene-tetramine Buffer Reagent Powder Pillow. Stopper. Invert several times to dissolve powder.

Note: *An orange to purple color develops if aluminum is present.*

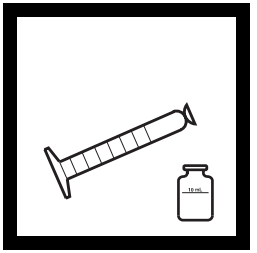


6. Put 1 drop of ECR Masking Reagent Solution into a 10-mL sample cell.



7. Pour 10 mL from the graduated mixing cylinder into the 10-mL sample cell. Swirl to mix (the blank).

Note: *The solution will start to turn yellow.*



8. Pour 10 mL from the graduated mixing cylinder into a second 10-mL sample cell. Swirl to mix (the prepared sample).

9. Wait 5 minutes, but not more than 10 minutes, before performing *step 10*.

Note: *The Pour-Thru cell cannot be used.*

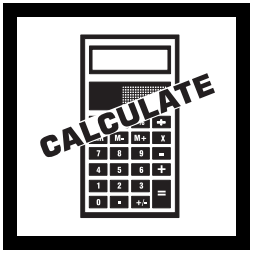
10. Zero the instrument with the blank, using the settings below.

DR/800
Program No. NA

DR/2010
Program No. 9
535 nm

DR/4000
Program No.1010
522 nm

11. Place the sample in the cell holder. Read the mg/L aluminum.



12. Use the equation below the aluminum ECR Sample and Analysis Volume Tables to calculate the true aluminum concentration.

Note: *If fluoride is present, it must be measured and the actual value determined using Table 2.*

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Al Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute to
0.05-1.3	40.0	8.00	20.0 mL
0.2-5.5	20.0	4.00	20.0 mL
0.8-22	10.0	2.00	20.0 mL
8.0-220	5.00	0.40	20.0 mL
80-2200	1.00	0.20	20.0 mL

Solids

Expected Al Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
4-110	0.500	8.00	20 mL
10-275	0.400	4.00	20 mL
27-730	0.300	2.00	20 mL
200-5500	0.200	0.40	20 mL
800-22000	0.100	0.20	20 mL

Calculation For Final Concentration:

$$\frac{A \times 2000}{B \times C} = \text{mg/L or mg/kg total Al}$$

A = mg/L reading from instrument

B = mL or g sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in a clean glass or plastic container. Preserve samples by adjusting the pH to 2 or less with nitric acid (about 1.5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 2.9 to 4.9 with 12.0 N Potassium Hydroxide Standard Solution and/or 1 N Potassium Hydroxide Solution. Correct the test result for volume additions.

Accuracy Check

Standard Solution Method

Prepare a 0.100 mg/L aluminum standard solution by pipetting 1.00 mL of Aluminum Standard Solution, 100 mg/L as Al^{3+}, into a 1000-mL volumetric flask. Dilute to the mark with deionized water. Prepare this solution daily. Perform the aluminum procedure as described above. The value should be 0.10 mg/L Al.

Or, using the TenSette Pipet, add 0.2 mL of solution from an Aluminum Voluette Ampule Standard Solution (50 mg/L as Al) into a 100-mL volumetric flask. Dilute to volume with deionized water. Perform the aluminum procedure as described above. The mg/L Al reading should be 0.10 mg/L.

Interferences

Table 1 lists common interferences and the amount of interference that can be expected.

Table 1 Interferences

Substance	Concentration	Error
Acidity	0-62 mg/L as $CaCO_3$	0%
Alkalinity	0-750 mg/L as $CaCO_3$	0%
Ca^{2+}	0-1000 mg/L as $CaCO_3$	0%
Cl^-	0-1000 mg/l	0%
Cr^{6+}	0.2 mg/L	-5% of reading
Cu^{2+}	2 mg/L	-5% of reading*
Fe^{2+}	0-4 mg/L	+ mg/L Fe^{2+} X 0.0075*
Fe^{3+}	0-4 mg/L	+ mg/L Fe^{3+} X 0.0075*
F^-	see Table 2	---
Mg^{2+}	0-1000 mg/L as $CaCO_3$	0%
Mn^{2+}	0-10 mg/L	0%
NO^{2-}	0-5 mg/L	0%
NO^{3-}	0-20 mg/L	0%
pH	2.9-4.9	0%
	7.5-11.5	0%
PO_4^{3-} (ortho)	4 mg/L	-5% of reading
SO_4^{2-}	0-1000 mg/L	0%
Zn^{2+}	0-10 mg/L	0%

* For more accurate results, correct results for the iron present. See the total iron procedure or measure with Hach Water Quality Total Iron test strips (Cat. No. 27453-25). Measure copper with Hach Water Quality Total Copper test strips (Cat. No. 27451-25).

A sample pH between about 4.9 and 7.5 causes dissolved aluminum to partially convert to colloidal and insoluble forms. This method measures much of that hard-to-detect aluminum without any pH adjustment as is necessary in some other methods.

Fluoride interference can be corrected by using *Table 2*.

Example:
If the fluoride concentration is 1.00 mg/L F^- and the ECR method gives a reading of 0.060 mg/L aluminum, what is the true mg/L aluminum concentration?
Answer: 0.183 mg/L

Intermediate values can be found by interpolation. Do not use correction graphs or charts found in other publications.

Table 2 True aluminum concentration (mg/L) vs. the instrument reading (mg/L) and fluoride concentration (mg/L) when the Eriochrome cyanine R method is used.

	Fluoride Concentration (mg/L)										
Instrument Reading (mg/L)	0.00	0.20	0.40	0.60	0.80	1.00	1.20	1.40	1.60	1.80	2.00
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.010	0.019	0.030	0.040	0.052	0.068	0.081	0.094	0.105	0.117	0.131
0.020	0.020	0.032	0.046	0.061	0.077	0.099	0.117	0.137	0.152	0.173	0.193
0.030	0.030	0.045	0.061	0.077	0.098	0.124	0.146	0.166	0.188	0.214	0.243
0.040	0.040	0.058	0.076	0.093	0.120	0.147	0.174	0.192	0.222		
0.050	0.050	0.068	0.087	0.109	0.135	0.165	0.188	0.217			
0.060	0.060	0.079	0.100	0.123	0.153	0.183	0.210	0.241			
0.070	0.070	0.090	0.113	0.137	0.168	0.201	0.230				
0.080	0.080	0.102	0.125	0.152	0.184	0.219					
0.090	0.090	0.113	0.138	0.166	0.200	0.237					
0.100	0.100	0.124	0.150	0.180	0.215						
0.120	0.120	0.146	0.176	0.209	0.246						
0.140	0.140	0.169	0.201	0.238							
0.160	0.160	0.191	0.226								
0.180	0.180	0.213									
0.200	0.200	0.235									
0.220	0.220										
0.240	0.240										

True Aluminum Concentration (mg/L) Al

Summary of Method

Eriochrome cyanine R combines with aluminum in a sample to produce an orange-red color. The intensity of color is proportional to the aluminum concentration.

REQUIRED REAGENTS

Description	Cat. No.
Aluminum Reagent Set (100 tests)	26037-00
Includes: (1)26038-49, (1) 26038-00, (1) 23801-23	

Description	Quantity Required Per Test	Unit	Cat. No.
ECR Reagent Powder	1 pillow	100/pkg	26038-49
Hexamethylenetetramine Buffer Reagent	1 pillow	100/pkg	26039-99
ECR Masking Reagent Solution	1 drop	25 mL	23801-23

REQUIRED APPARATUS

Cylinder, 25 mL, mixing graduated	1	each	20886-40

Select one or more based an sample amount and analysis volume:

Pipet, volumetric, Class A, 8.0 mL	1	each	14515-08
Pipet, volumetric, Class A, 4.0 mL	1	each	14515-04
Pipet, volumetric, Class A, 2.0 mL	1	each	14515-36
TenSette® Pipet, 0.1-1.0 mL	1	each	19700-01
Pipet Tips, TenSette® Pipet	varies	50/pkg	21856-96

OPTIONAL REAGENTS

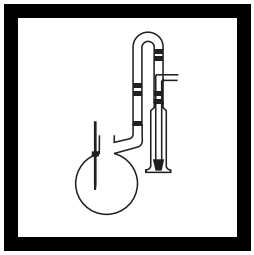
Aluminum Reagent Set for 25 mL samples	100 tests	24434-00
Aluminum Standard Solution, 100 mg/L	100 mL	14174-42
Aluminum Standard Solution, Voluette™ ampule, 50 mg/L as Al, 10 mL	16/pkg	14792-10
Bromphenol Blue Indicator Solution	100 mL MDB	14552-32
Hydrochloric Acid Solution, 6 N (1:1)	500 mL	884-49
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Potassium Hydroxide Solution, 1 N	50 mL SCDB	23144-26
Potassium Hydroxide Standard Solution, 12.0 N	100 mL	230-32
Potassium Hydroxide Standard Solution, 12.0 N	500 mL	230-49
SPADNS Fluoride Reagent AccuVac® Ampuls	25/pkg	25060-25
Sulfuric Acid Standard Solution, 5.25 N	100 mL MDB	2449-32
Water, deionized	4 L	272-56
Water Quality Test Strips, total iron, 0-5 ppm	25/pkg	27453-25
Water Quality Test Strips, total copper	25/pkg	27451-25

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Ampule Breaker Kit	each	21968-00
Brush	each	690-00
Cylinder, graduated, mixing, 50 mL	each	1896-41
Flask, Erlenmeyer, glass, 125 mL	each	505-43
Flask, volumetric, 100 mL	each	14574-42
Flask, volumetric, 1000 mL	each	14574-53
Fluoride Combination Electrode	each	50265-00
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
pH/ISE Meter, *sens**ion**™2*, portable	each	51725-10
Pipet Filler, safety bulb	each	14651-00
Pipet, serological, 2 mL	each	532-36
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, TenSette®, 1.0 to 10.0 mL	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	50/pkg	21997-96
Thermometer, -10° to 110 °C	each	1877-01

ARSENIC

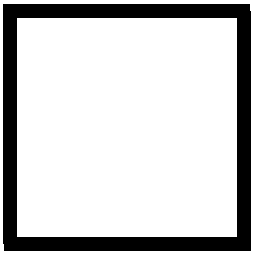
Silver Diethyldithiocarbamate Method*
USEPA accepted for reporting with distillation**
Range: 0–0.200 mg/L



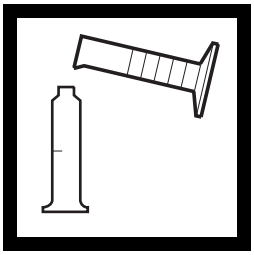
1. Prepare the Hach distillation apparatus for arsenic recovery. Place it under a fume hood to vent toxic fumes.

Note: *See the Hach Distillation Manual for assembly instructions.*

Note: *Requires a user-entered calibration for Hach spectrophotometers; see the specific procedure manuals for instructions.*

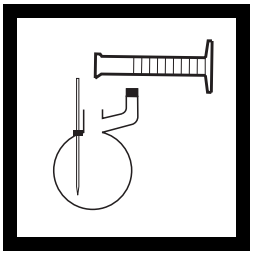


2. Dampen a cotton ball with 10% Lead Acetate Solution. Place it in the gas scrubber. Be certain the cotton seals against the glass.



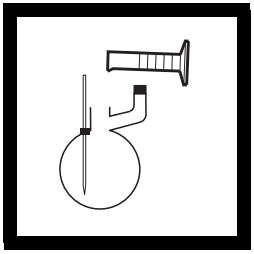
3. Measure 25 mL of prepared arsenic absorber solution into the cylinder/gas bubbler assembly with a graduated cylinder. Attach it to the distillation apparatus.

Note: *Prepare the arsenic absorber solution as directed under Reagent Preparation below.*

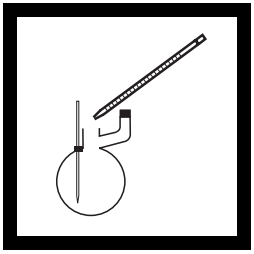


4. Measure 250 mL of sample into the distillation flask using a graduated cylinder.

5. Turn on the power switch. Set the stir control to 5. Set the heat control to 0.

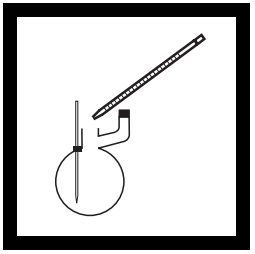


6. Measure 25 mL of concentrated ACS hydrochloric acid into the flask using a graduated cylinder.



7. Measure 1 mL of Stannous Chloride Solution into the flask.

Note: *Use a serological pipet to measure the solution.*

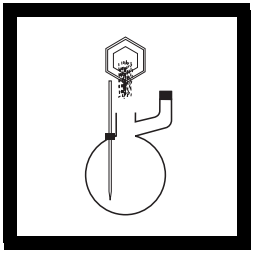


8. Add 3 mL of Potassium Iodide Solution to the flask. Cap.

Note: *Use a serological pipet to measure the solution.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Equivalent to USEPA Method 206.4 for wastewater

9. Start a 15 minute reaction period.



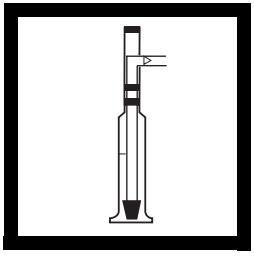
10. When the timer beeps, add 6.0 g of 20-mesh zinc to the flask. Cap immediately.

11. Set the heat control to 3.

Start a second 15-minute reaction period.

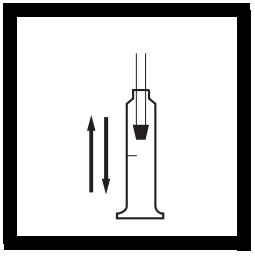
12. When the timer beeps, set the heat control to 1.

Start a third 15-minute reaction period.



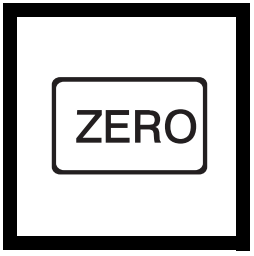
13. When the timer beeps, turn off the heater.

Remove the cylinder/gas bubbler assembly as a unit.



14. Rinse the gas bubbler by moving it up and down in the arsenic absorber solution.

15. Fill a dry sample cell with unreacted arsenic absorber solution (the blank). Stopper. Place it into the cell holder.



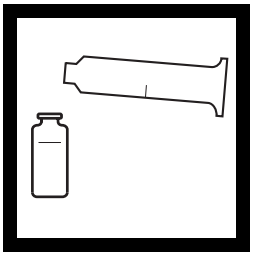
16. Zero the instrument with the blank, using the settings below.

DR/800s- NA

DR/2010
Program No. (User entered numbered)
520 nm

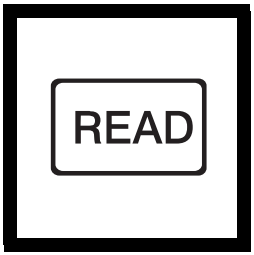
DR/4000
Program No. (User-entered number)
520 nm

Note: *See the specific spectrophotometer procedure or instrument manual for calibration instructions.*



17. Pour the reacted arsenic absorber solution into a sample cell (the prepared sample). Stopper.

Note: *If the solution volume is less than 25 mL, add pyridine to bring the volume to exactly the 25-mL mark. Swirl to mix.*



18. Place the sample in the cell holder. Read the mg/L arsenic.

Sampling and Storage

Collect samples in acid washed glass or plastic bottles. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature. Correct the test result for volume additions.

Preparing Arsenic Absorber Solution

Prepare the arsenic absorber solution as follows:

1. Weigh 1.00 g of silver diethyldithiocarbamate on an analytical balance.

2. Transfer the powder to a 200-mL volumetric flask. Dilute to volume with pyridine. Use pyridine only in a fume hood.

3. Mix well to dissolve. Store the reagent, tightly sealed, in an amber bottle. The reagent is stable for one month if stored in this manner. Larger volumes of reagent can be prepared if the reagent is used within one month.

Accuracy Check

a. Prepare a 10.0-mg/L working standard by pipetting 1.0 mL of Arsenic Standard, 1000-mg/L as AS, into a 100-mL volumetric flask. Dilute to volume with deionized water. Mix well.

b. Prepare a 0.12-mg/L arsenic standard solution by diluting 3.0 mL of the working standard into a 250-mL volumetric flask. Dilute to volume with deionized water. Mix well.

c. Perform the arsenic procedure described, using the working standard as a sample. The results should be 0.12 mg/L As.

Interferences

Antimony salts may interfere with color development.

Summary of Method

Arsenic is reduced to arsine gas by a mixture of zinc, stannous chloride, potassium iodide and hydrochloric acid in a specially equipped distillation apparatus. The arsine is passed through a scrubber containing cotton saturated with lead acetate and then into an absorber tube containing silver diethyldithiocarbamate in pyridine. The arsenic reacts to form a red complex which is read colorimetrically. This procedure requires a manual calibration.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Arsenic Standard Solution, 1000 mg/L As	varies	100 mL	14571-42
Hydrochloric Acid, ACS	25 mL	500 mL	134-49
Lead Acetate Solution, 10%	1 mL	100 mL	14580-42
Potassium Iodide Solution, 20%	3 mL	100 mL	14568-42
Pyridine	50 mL	500 mL	14469-49
Silver Diethyldithiocarbamate	1 g	25 g	14476-24
Stannous Chloride Solution	1 mL	100 mL	14569-42
Zinc, 20-mesh, ACS	6 g	454 g	795-01

REQUIRED APPARATUS

Balance, analytical	1	each	22310-00
Boat, weighing	2	500/pkg	21790-00
Bottle, amber, 237 mL	1	6/pkg	7144-41
Cap, polypropylene	1	6/pkg	21667-06
Cotton Balls	1	100/pkg	2572-01
Cylinder, graduated, 25 mL	2	each	508-40
Cylinder, graduated, 250 mL	1	each	508-46
Distillation Apparatus Arsenic Accessories	1	set	22654-00
Distillation Apparatus General Purpose Accessories	1	set	22653-00
Flask, volumetric, 100 mL	1	each	14574-42
Flask, volumetric, 200 mL	1	each	14574-45
Flask, volumetric, 250 mL	4	each	14574-46
Pipet Filler, safety bulb	1	each	14651-00
Stopper, hollow, poly, No. 0	2	6/pkg	14480-00
OR			
Stopper, hollow, poly, No. 1	2	6/pkg	14480-20

Select one based on available voltage:

Distillation Apparatus Heater, 115 Vac, 60 Hz		each	22744-00
Distillation Apparatus Heater, 230 Vac, 50 Hz		each	22744-02

OPTIONAL REAGENTS

Description	Unit	Cat. No.
Hydrochloric Acid, ACS	2.5 L	134-06
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Pyridine, ACS	4 L	14469-17
Water, deionized	4 L	272-56

OPTIONAL APPARATUS

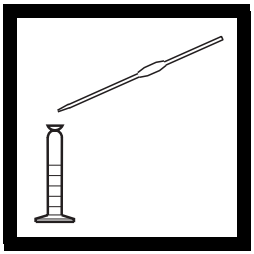
pH Meter, *sens**ion***™*1*, portable	each	51700-10
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipet, serological, 2 mL	each	532-36
Pipet, serological, 5 mL	each	532-37
Pipet, TenSette®, 1.0 to 10.0 mL	each	19700-10
Pipet Tips, for 19700-01	50/pkg	21997-96
Pipet Tips, for 19700-01	250/pkg	21997-25
Pipet, volumetric, 1 mL	each	14515-35
Pipet, volumetric, 2 mL	each	14515-36
Pipet, volumetric, 3 mL	each	14515-03
Pipet, volumetric, 4 mL	each	14515-04

CADMIUM, TOTAL

Dithizone Method*
Range: Liquids- 0.05–4000 mg/L; Solids- 5–40000 mg/kg

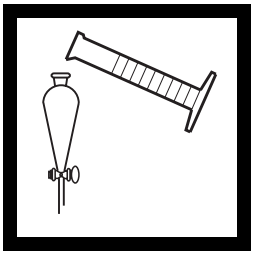
1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

Note: *If sample cannot be analyzed shortly after collection, see* Sampling and Storage *following these steps.*



2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 250-mL mixing cylinder. Dilute to the mark with deionized water. If the aliquot is more than 5 mL, adjust the pH according to the instructions following the digestion method

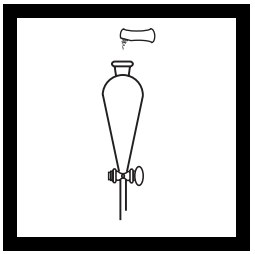
Note: *Clean all glassware with Nitric Acid Solution, 1:1. Rinse with deionized water.*



3. Fill a 250-mL graduated cylinder to the 250-mL mark with sample. Pour the sample into a 500-mL separatory funnel.

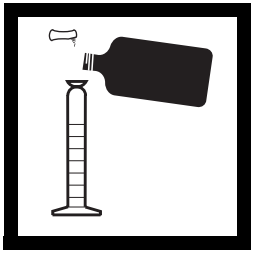
Note: *Cloudy and turbid samples may require filtering with a glass membrane filter before running test.*

Note: *For best results, run a deionized water blank through the entire procedure. Subtract the blank value from the test results. Repeat for every new lot of DithiVer Reagent Powder Pillows.*



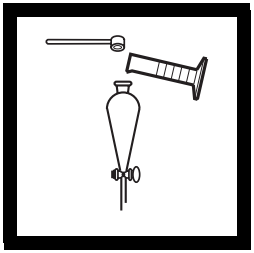
4. Add the contents of one Buffer Powder Pillow, citrate type for heavy metals. Stopper the funnel. Shake to dissolve.

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*



5. Add 30 mL of chloroform to a 50-mL mixing graduated cylinder. Add the contents of one DithiVer Metals Reagent Powder Pillow. Stopper. Invert repeatedly to mix (DithiVer solution).

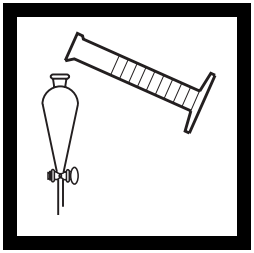
Note: *Use adequate ventilation. The DithiVer powder will not all dissolve. See DithiVer Solution Preparation and Storage following these steps.*



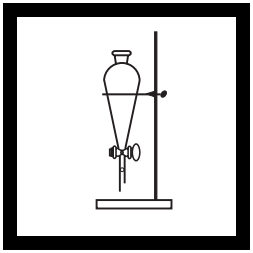
6. Add 20 mL of 50% Sodium Hydroxide Solution and then a 0.1-g scoop of potassium cyanide to the funnel. Shake vigorously for 15 seconds. Remove the stopper and let stand for one minute.

Note: Potassium cyanide is a deadly poison. *Avoid ingestion, inhalation, or contact with the skin. Do not bring into contact with acids.*

Note: *Spilled reagent will affect test accuracy and is hazardous.*

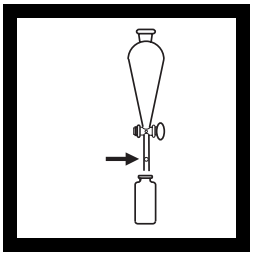


7. Add 30 mL of the DithiVer solution to the 500-mL separatory funnel. Stopper, invert, and open stopcock to vent. Close the stopcock and shake funnel once or twice; vent again. Close the stopcock and shake the funnel vigorously for 60 seconds.



8. Let the funnel stand undisturbed for roughly one minute.

Note: *The bottom (chloroform) layer will be pink if cadmium is present*



9. Insert a cotton plug the size of a pea into the delivery tube of the funnel and slowly drain the bottom (chloroform) layer into a dry 25-mL sample cell (the prepared sample). Cap.

Note: *The cadmium-dithizone complex is stable for hours if the sample cell is capped tightly and out of direct sunlight.*

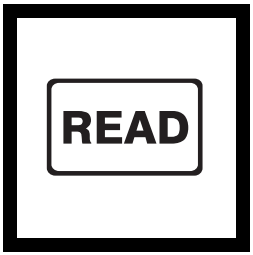
10. Fill a dry 25-mL sample cell with chloroform (the blank). Cap.

11. Zero the instrument with the blank, using the settings below.

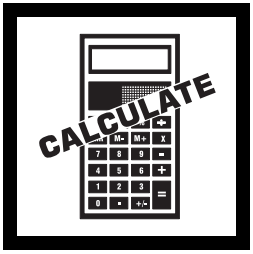
DR/800s
NA

DR/2010
Program No. 60
515 nm

DR/4000
Program No.1350
515 nm



12. Place the sample in the cell holder. Read the µg/L cadmium.



13. Use the equation below the cadmium dithizone Sample and Analysis Volume Tables to calculate the true cadmium concentration.

Note: *For solid samples, express the concentration as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Cd Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.05-2.5	40.0	20.0	250 mL
0.2-10	20.0	10.0	250 mL
1-40	10.0	5.00	250 mL
10-400	5.00	1.00	250 mL
100-4000	1.00	0.50	250 mL

Solids

Expected Cd Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
5-200	0.500	20.0	250 mL
13-500	0.400	10.0	250 mL
35-1300	0.300	5.00	250 mL
250-10000	0.200	1.00	250 mL
1000-40000	0.100	0.50	250 mL

Calculation For Final Concentration:

$\frac{A \times 25}{B \times C}$ = mg/L or mg/kg Total Cd or

$\frac{A \times 25000}{B \times C}$ = µg/kg or µg/L Total Cd

A = µg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Safety Precautions

Perform the entire procedure in a fume hood if possible. Using gloves that are resistant to chloroform solutions (i.e. VITON) and safety goggles is recommended.

Sampling and Storage

Collect samples in an acid-cleaned glass or plastic container. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Store preserved samples up to six months at room temperature. Adjust the pH to 2.5 with 5.0 N sodium hydroxide before analysis. Correct the test result for volume additions.

Dithiver Solution Preparation and Storage

Store DithiVer powder pillows away from light and heat. A convenient way to prepare this solution is to add the contents of 16 DithiVer Metals Reagent Powder Pillows to a 500-mL bottle of chloroform and invert several times until well mixed (carrier powder may not dissolve). Store dithizone solution in an amber glass bottle. This solution is stable for 24 hours.

Accuracy Check

Standard Additions Method

a. Perform the cadmium method and note the analysis volume used and the µg/L of the sample in *step 12*.

b. Pipet the analysis volume into three 250-mL graduated cylinders.

c. Snap the neck off a Cadmium Voluette Ampule Standard Solution, 25 mg/L Cd.

d. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to three cylinders. Dilute to 250 mL. Mix each thoroughly.

e. Analyze each sample as described above. The cadmium concentration should increase 10 µg/L for each 0.1 mL of standard added compared to the value in step a. If these increases do not occur, an interference is likely.

Standard Solution Method
Prepare a 5.0-mg/L cadmium standard solution by pipetting 5.00 mL of Cadmium Standard Solution, 100-mg/L Cd, into a 100-mL volumetric flask. Dilute to the mark with deionized water and mix thoroughly. Prepare a fresh solution daily.

Pipet 2.00 mL of the 5.0-mg/L cadmium standard solution into 248 mL of deionized water in a 500-mL separatory funnel. This is a 40 µg/L cadmium solution. Perform the cadmium test as described in the procedure.

Interferences

Strong oxidants will destroy the dithizone indicator and cause high blanks and/or low results.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.

The following do not interfere:

Aluminum	Lead
Antimony	Magnesium
Arsenic	Manganese
Calcium	Nickel
Chromium	Tin
Cobalt	Zinc
Iron	

The following interfere causing high results when present in concentrations exceeding those listed below:

Copper	2 mg/L*
Bismuth	80 mg/L
Mercury	all levels
Silver	2 mg/L

* Measure copper levels using Hach Water Quality Total Copper test strips (Cat. No. 27451-25).

Eliminate interference from these metals by the following treatment, beginning after *step 5*.

a. Measure about 5 mL of the DithiVer solution into the separatory funnel. Stopper the funnel, invert and open the stopcock to vent. Close the stopcock and shake the solution vigorously for 15 seconds. Allow the funnel to stand undisturbed until the layers separate (about 30 seconds). A yellow, red, or bronze color in the bottom (chloroform) layer confirms the presence of interfering metals. Draw off and discard the bottom layer.

b. Repeat extraction with fresh 5 mL portions of the DithiVer solution (discarding the bottom layer each time) until the bottom layer shows a pure dark green color for three successive extracts. Extractions can be repeated several times without appreciably affecting the amount of cadmium in the sample.

c. Extract the solution with several 2 or 3 mL portions of pure chloroform to remove any remaining DithiVer, again discarding the bottom layer each time.

d. Continue with *step 6*.

e. In *step 7*, substitute 28.5 mL of DithiVer solution for the 30 mL.

f. Continue with *step 8*.

Pollution Prevention and Waste Management

Both chloroform (D002) and cyanide (D003) solutions are regulated as hazardous wastes by the Federal RCRA. Do **not** pour these solutions down the drain. Collect chloroform solutions and the cotton plugs used in the delivery tube of the separatory funnel for disposal with laboratory solvent waste. Be sure cyanide solutions are stored in a caustic solution with a pH >11 to prevent release of hydrogen cyanide gas.

Summary of Method

The dithizone method determines cadmium in water and wastewater. The DithiVer metals reagent is a stable powder form of dithizone. Cadmium ions in basic solution react with dithizone to form a pink to red cadmium-dithizonate complex, which is extracted with chloroform.

REQUIRED REAGENTS

Description	Cat No.
Cadmium Reagent Set (100 Tests)	22422-00

Includes: (1) 14202-99, (1) 14458-17, (1) 12616-99, (1) 767-14, (4) 2180-49

Description	Quantity Required Per Test	Unit	Cat. No.
Buffer Powder Pillows, citrate for heavy metals	1 pillow	100/pkg	14202-99
Chloroform, ACS	30 mL	4 L	14458-17
DithiVer® Metals Reagent Powder Pillows	1 pillow	100/pkg	12616-99
Potassium Cyanide, ACS	0.1 g	113 g	767-14
Sodium Hydroxide Solution, 50%	20 mL	500 mL	2180-49
Cotton Balls, absorbent	1	100/pkg	2572-01

REQUIRED APPARATUS

Clippers, for opening pillows	1	each	968-00
Cylinder, graduated, 25 mL	1	each	508-40
Cylinder, graduated, 250 mL	1	each	508-46
Cylinder, mixing, graduated, 50 mL	1	each	1896-41
Funnel, separatory, 500 mL	1	each	520-49
Spoon, measuring, 0.1 g	1	each	511-00
Support Ring, 4 in.	1	each	580-01
Support Stand, 5 x 8 in	1	each	563-00

Select one or more of the following based on sample and analysis volume:

Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20

OPTIONAL REAGENTS

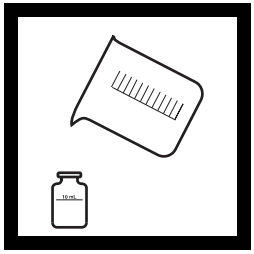
Description	Unit	Cat. No.
Cadmium Standard Solution, 100 mg/L as Cd	100 mL	14024-42
Cadmium Standard Solution, Voluette™ ampule, 25 mg/L Cd, 10 mL	16/pkg	14261-10
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	2450-26
Water, deionized	4 L	272-56
Water Quality Test Strips, total copper	25/pkg	27451-25

OPTIONAL APPARATUS

Ampule Breaker Kit	each	21968-00
Cylinder, graduated, 5 mL	each	508-37
Filter Discs, glass, 47 mm	100/pkg	2530-00
Flask, Erlenmeyer, 500 mL	each	505-49
Flask, filtering, 500 mL	each	546-49
Flask, volumetric, 100 mL	each	547-42
Membrane Filter Holder, 500 mL, for 47 mm filter	each	2340-00
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable	each	51700-10
Pipet, serological, 2 mL	each	532-36
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 2.00 mL	each	14515-36
Pipet Filler, safety bulb	each	14651-00
Stopper, hollow, poly, Size No. 2	6/pkg	14480-01

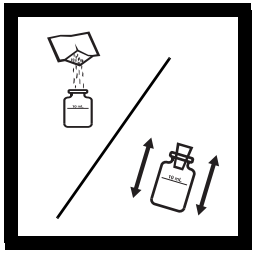
CHLORINE, TOTAL

DPD Method* USEPA accepted for reporting wastewater analysis**
Range: 0 to 2.00 mg/L



1. Fill a 10-mL sample cell with 10 mL of sample.

Note: *Samples must be analyzed immediately and cannot be preserved for later analysis.*

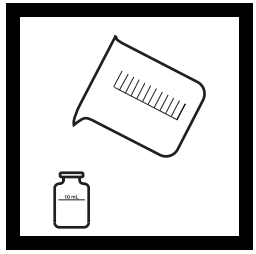


2. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell. Stopper the sample cell and shake for 20 seconds. Remove the stopper.

Note: *Shaking dissipates bubbles which may form.*

3. Begin a three-minute reaction period.

Note: *A pink color will develop if chlorine is present.*



4. When the timer beeps fill another sample cell (the blank) with 10 mL of sample.

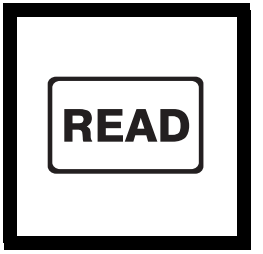
* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Procedure is equivalent to USEPA Method 330.5 for wastewater

5. Place the blank into the cell holder. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 9

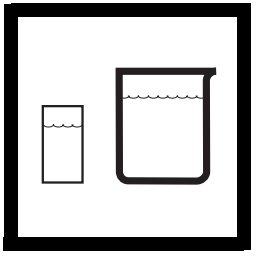
DR/2010
Program No. 80
530 nm

DR/4000
Program No.1450
530 nm



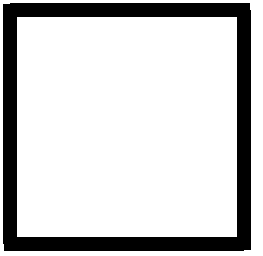
6. Within three minutes after the timer beeps, place the sample into the cell holder. Read the mg/L chlorine.

Using AccuVac Ampuls



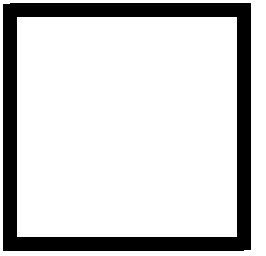
1. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50-mL beaker.

Note: *Samples must be analyzed immediately and cannot be preserved for later analysis.*



2. Fill a DPD Total Chlorine Reagent AccuVac Ampul with sample.

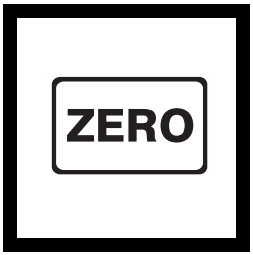
Note: *Keep the tip immersed while the ampul fills completely.*



3. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: *A pink color will form if chlorine is present.*

4. Begin a three-minute reaction period. Insert the AccuVac Adapter into the instrument during this period.



5. Place the blank into the adapter. Zero the instrument with the blank, using the settings below.

DR/800
Program No. 11

DR/2010
Program No. 85
530 nm

DR/4000
Program No. 1460
530 nm

6. Within three minutes after the timer beeps, place the sample in the adapter. Read the mg/L chlorine.

Note: *If the sample temporarily turns yellow after sample addition, or shows* ***OVER-RANGE****, dilute a fresh sample and repeat the test. A slight loss of chlorine may occur during dilution. Multiply the result by the appropriate dilution factor.*

Sampling and Storage

Analyze samples for chlorine **immediately** after collection. Chlorine is a strong oxidizing agent, and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. **Pretreat glass** sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least one hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample containers for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample containers for free and total chlorine determinations.

A common error in testing for chlorine is introduced when a representative sample is not obtained. If sampling from a tap, let the water flow for at least five minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark. Perform the chlorine analysis immediately.

Accuracy Check

Standard Additions Method (using Powder Pillows)

a. Perform a sample analysis and record the result.

b. Snap the top off a LR Chlorine PourRite Ampule Standard Solution, 25-30 mg/L Cl_2.

c. Use a TenSette Pipet to add 0.1 mL of the standard to the reacted sample (this is the spiked sample). Swirl to mix.

d. Place the spiked sample into the instrument and read the spiked sample result.

e. Calculate the equivalent concentration of mg/L chlorine added to the sample:

$$\text{mg/L Chlorine} = \frac{0.1(\text{vol. standard added}) \times \text{Certificate value}(\text{mg/L } Cl_2)}{10.1(\text{sample} + \text{standard volume})}$$

f. The spiked sample result (*step d*) should reflect the analyzed sample result (*step a*) + the added, calculated mg/L Cl_2 (*step e*).

g. If this increase does not occur, an interference is likely.

Standard Additions Method (using AccuVac Ampuls)

a. Snap the top off a LR Chlorine PourRite Ampule Standard Solution, 25-30 mg/L Cl_2.

b. Use a graduated cylinder to measure 25 mL of sample into each of two beakers.

c. Use a TenSette Pipet to add 0.2 mL of the standard to one of the beakers (this is the spiked sample). Swirl to mix.

d. Fill a DPD Total Chlorine AccuVac completely from each beaker.

e. Analyze the spiked and unspiked sample as described in the procedure.

f. Calculate the equivalent concentration of mg/L chlorine added to the sample:

$$\text{mg/L Chlorine} = \frac{0.2(\text{vol. standard added}) \times \text{Certificate value}(\text{mg/L } Cl_2)}{25.2(\text{sample + standard volume})}$$

g. The spiked sample result should reflect the analyzed sample result + the added, calculated mg/L Cl_2 (*step f*).

h. If this increase does not occur, an interference is likely.

Interferences

Samples containing more than **300 mg/L alkalinity*** or **150 mg/L acidity** as $CaCO_3$ may not develop the full amount of color, or it may instantly fade. Neutralize these samples to a pH of 6 to 7 with 1 N sulfuric acid, or 1 N sodium hydroxide. Determine the amount required on a separate 10 mL sample. Add the same amount to the sample to be tested. Correct for volume additions.

Bromine, **iodine**, **ozone**, **oxidized manganese**, and **chromium** also may react and read as chlorine. To compensate for the effects of oxidized manganese or chromium, adjust the pH to between 6 and 7 as described above; then add 3 drops of potassium iodide, 30 g/L, to 25 mL of sample, mix, and wait one minute. Add 3 drops of sodium arsenite, 5 g/L, and mix. Analyze 10 mL of this sample as described above. (If chromium is present, allow exactly

* Monitor alkalinity with Hach Water Quality Alkalinity Test Strips (Cat. No. 27448-50).

the same reaction period with the DPD for both analyses.) Subtract the result of this test from the original analysis to obtain the correct chlorine result.

DPD Total Chlorine Reagent Powder Pillows and AccuVac Ampuls contain a buffer formulation which will withstand high levels of hardness (at least 1000 mg/L) without interference.

Summary of Method

Chlorine can be present in water as free available chlorine and as combined available chlorine. Both forms can exist in the same water and be determined together as the total available chlorine. Free chlorine is present as hypochlorous acid and/or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives.

The combined chlorine oxidizes iodide in the reagent to iodine. The iodine reacts with DPD (N, N-diethyl-p-phenylenediamine) along with free chlorine present in the sample to form a red color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run the free chlorine and total chlorine tests. Subtract the results of the free chlorine test from the results of the total chlorine test to obtain combined chlorine.

REQUIRED REAGENTS (Using Powder Pillows)

Description	Quantity Required Per Test	Unit	Cat. No.
DPD Total Chlorine Reagent Powder Pillows	1 pillow	100/pkg	21056-69

REQUIRED REAGENTS (Using AccuVac® Ampuls)

DPD Total Chlorine Reagent AccuVac® Ampuls	1 ampul	25/pkg	25030-25

REQUIRED APPARATUS (Using Powder Pillows)

Stopper, hollow, LDPE, No. 2 (fits 10 & 25-mL cells)	1	6/pkg	14480-01

REQUIRED APPARATUS (Using AccuVac® Ampuls)

Beaker, 50 mL	1	each	500-41
Vial, zeroing	1	each	21228-00

OPTIONAL REAGENTS

Chlorine Standard Solution, PourRite® ampule, 25-30 mg/L	20/pkg		26300-20
DPD Total Chlorine Reagent w/dispensing cap	250 tests		21056-29
Potassium Iodide Solution, 30 g/L	100 mL*	MDB	343-32
Sodium Arsenite, 5 g/L	100 mL*	MDB	1047-32
Sodium Hydroxide Standard Solution, 1 N	100 mL*	MDB	1045-32
Sulfuric Acid Standard Solution, 1 N	100 mL*	MDB	1270-32
Water, deionized	4 L		272-56

OPTIONAL APPARATUS

AccuVac® Ampul Snapper Kit	each		24052-00
Ampule Breaker Kit	each		24846-00
Cylinder, graduated, 25 mL	each		508-40
pH Indicator Paper, 1 to 11 pH units	5 rolls/pkg		391-33
pH Meter, *sens**ion***™*1*, portable	each		51700-10
Pipet, TenSette®, 0.1 to 1.0 mL	each		19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg		21856-96

* Marked Dropper Bottle - contact Hach for larger sizes.

CHLORINE, TOTAL, ULTRA LOW RANGE

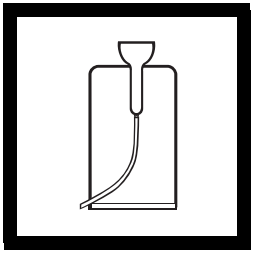
DPD Method for Treated Wastewater* USEPA Accepted for reporting wastewater analyses**
Range: 0 to 500 µg/L

1. Set up the spectrophotometer with the following settings:

DR/800
Program No. NA

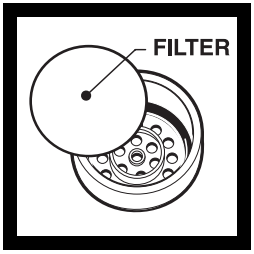
DR/2010
Program No. 86
515 nm

DR/4000
Program No.1490
515 nm



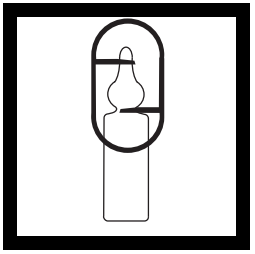
2. Install the Pour-Thru or Flow-Thru Cell and flush with 50 mL of deionized water.

Note: *See Treating Analysis Labware section.*



3. Unscrew the cap from the OriFlo™* Filter plunger assembly. Be sure the O-ring is properly seated in the cap. Install a new 3-micron filter into the cap well. Wet the filter with a few drops of deionized water. Re-assemble and hand-tighten the cap onto the plunger.

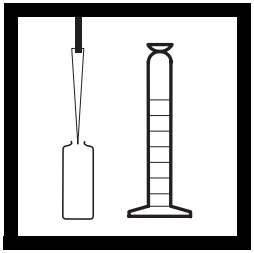
Note: *To obtain accurate results, use a new 3-µm filter (specified) for each test.*



4. Break open one ampule of ULR Chlorine Buffer Solution.

Note: *The Ampule Breaker is a convenient, safe way to open ampules.*

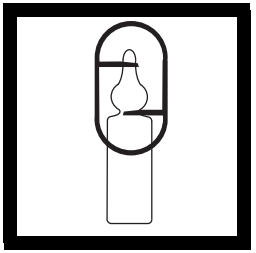
* U.S. Patent 5,362,650. U.S. Patent No. for the OriFloFilter is 5,549,816
** Procedures is equivalent to standard Method 4500-Cl D for wastewater.



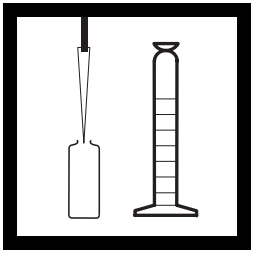
5. Using a TenSette Pipet with a clean tip, transfer 1.0 mL of buffer to a clean, treated 50-mL graduated mixing cylinder.

Note: *See Treating Analysis Labware section.*

Note: *The ampules contain more than 1.0 mL for ease of reagent transfer. Discard excess reagent.*

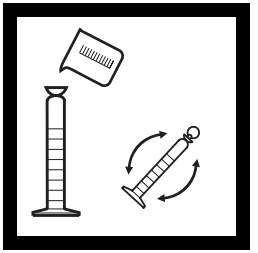


6. Break open one ampule of DPD Indicator Solution for Ultra Low Range Chlorine.



7. Using a TenSette Pipet with a clean tip, transfer 1.0 mL of indicator from the ampule to the cylinder. Swirl to mix reagents.

Proceed with *step 9* within one minute.

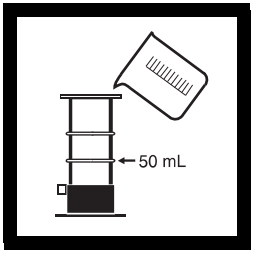


8. Avoiding extra agitation, carefully fill the cylinder to the 50-mL mark with sample. Stopper. Gently invert twice to mix (the prepared sample).

9. Begin a three minute reaction period.

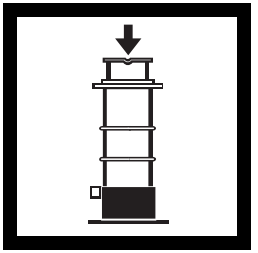
Note: *Measure the sample absorbance 3-6 minutes after mixing the sample and reagents. If less than three minutes elapses, reaction with chloramines may be incomplete. A reading after 6 minutes may result in higher blank values*

Note: *Perform steps 9-14 during the reaction period.*

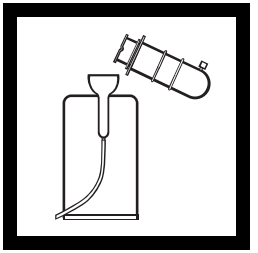


10. During the three-minute period, push the valve button on the OriFlo Filter's barrel assembly to the "closed" position. Place the barrel assembly into its stand. Pour about 50 mL of the original sample into the barrel.

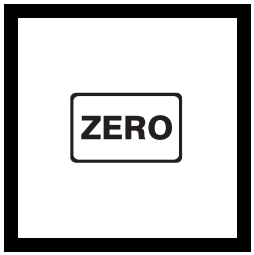
Note: *The lower ring on the barrel assembly represents about 50 mL.*



11. Insert the plunger into the barrel and slowly push the plunger down with even pressure until the plunger is fully seated.

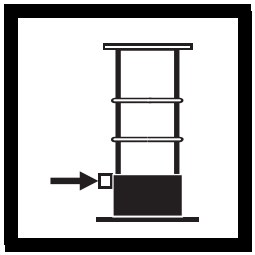


12. Pour the filtered sample from the plunger reservoir into the Pour-Thru or Flow-Thru Cell.



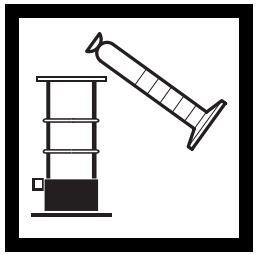
13. If using a DR/2010: press **ZERO** after the flow stops.

If using a DR/4000: press the soft key under ***ZERO***.

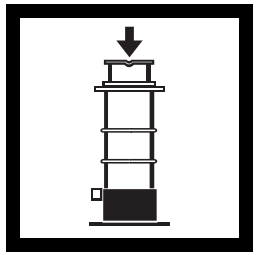


14. Pull the barrel's valve button to the open position (1). Pull the plunger up to separate it from the barrel assembly (2). Discard the rest of the unfiltered sample.

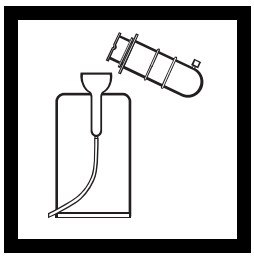
Note: *For very turbid samples, install a new membrane. Or, use a second OriFlo Filter unit with a new membrane filter installed.*



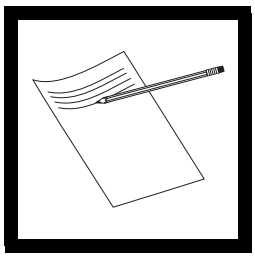
15. Push the barrel valve to the closed position and return the barrel to its stand. When the timer beeps, pour the contents of the graduated mixing cylinder into the barrel.



16. Insert the plunger into the barrel and slowly push the plunger down with even pressure, until the plunger is fully seated.



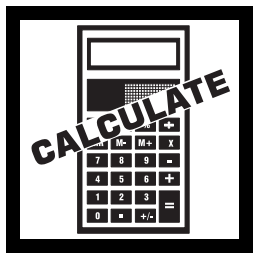
Pour the filtered, reacted sample from the plunger reservoir into the Pour-Thru or Flow-Thru Cell.



17. After the flow stops and the reading stabilizes, the result in µg/L Cl_2 will be displayed. Record the result.

Note: *If a dechlorination agent such as sulfite or sulfur dioxide is present in the sample, the sample result, corrected for the reagent blank, will read "0" or a slightly negative value.*

18. Flush the Pour-Thru or Flow-Thru Cell with at least 50 mL of deionized water immediately after use.

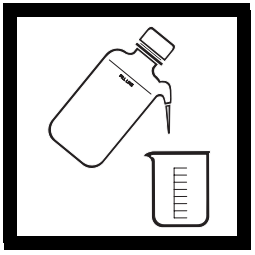


19. Determine the reagent blank using the procedure on the next page. Subtract the reagent blank value (in µg/L) from the value obtained in *step 18*.

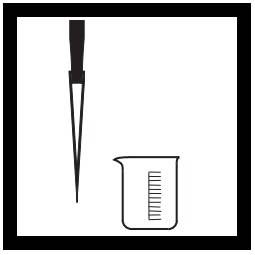
Note: *Determine the reagent blank value for a combined lot of Indicator/Buffer at least once a day.*

Determining the Reagent Blank Value

1. Set up the spectrophotometer as described in *step 1* on page *101*.



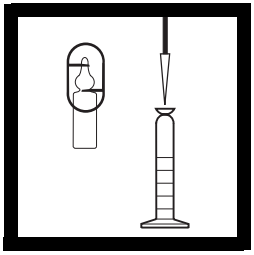
2. Collect about 100 mL deionized or tap water in a clean 250-mL beaker.



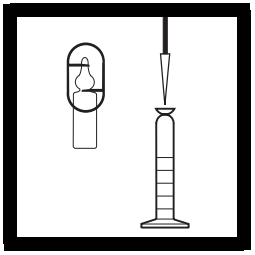
3. Using a TenSette Pipet, add 1.0 mL Blanking Reagent to the beaker. Swirl several times to mix.

Note: *The Blanking Reagent removes chlorine from the sample.*

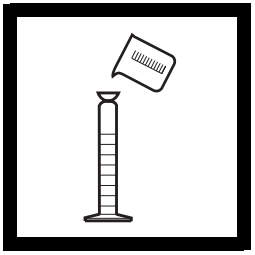
4. Begin a five-minute reaction period.



5. After the timer beeps, break open one ampule of ULR Chlorine Buffer Solution. Using a TenSette Pipet with a clean tip, transfer 1.0 mL of buffer from the ampule to a clean 50-mL graduated mixing cylinder.

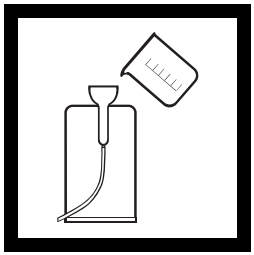


6. Break open one ampule of ULR DPD Indicator Solution. Using a TenSette Pipet with a clean tip, transfer 1.0 mL of indicator from the ampule to the cylinder. Swirl to mix the reagents. Proceed with *step 7* within 1 minute.

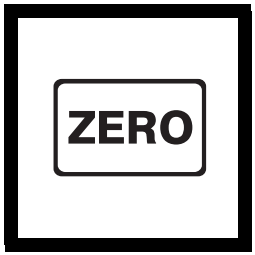


7. Fill the cylinder to the 50-mL mark with the dechlorinated water from *step 3*. Cap and invert twice to mix. Save the remaining water for *step 9*.

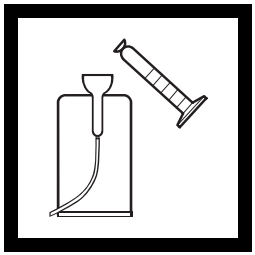
8. Begin a three-minute reaction period.



9. During the reaction period, flush the Pour-Thru or Flow-Thru Cell with the remainder of the original dechlorinated water from *step 7*.



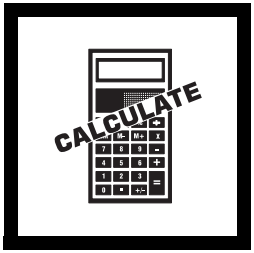
10. When the flow stops, press **ZERO** (or the soft key under ***ZERO***).



11. When the timer beeps, pour the contents of the cylinder into the Pour-Thru or Flow-Thru Cell.

12. After the flow stops, the reagent blank value will be displayed in µg/L Cl_2. Record this value.

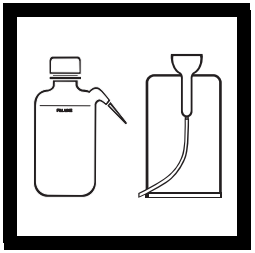
Note: *If using a DR/4000, store the blank value by pressing* **OPTIONS (MORE)**, *then* **BLANK (OFF)**. *Enter the value and press* ***ENTER****. Repeat for every lot of reagent.*



13. Use this value to correct the sample result obtained.

Note: *The DR/4000 will automatically correct for the reagent blank if entered as directed in the Step 12 note.*

Note: *The reagent blank value is normally less than 5 µg/L. If it is greater than 5 µg/L, interfering substances may be present in the blanking water or the DPD Indicator may be developing some color. Blanks of up to 5 µg/L may be used.*



14. Flush the Pour-Thru or Flow-Thru Cell with at least 50 mL deionized water immediately after use.

Sample Collection and Storage

Analyze samples for chlorine **immediately** after collection. Chlorine is a strong oxidizing agent, and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature, and salinity influence decomposition of chlorine in water.

Avoid plastic containers since these may have a large chlorine demand. **Pretreat glass** sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.

Do not use the same sample containers for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample containers for free and total chlorine determinations.

A common error in testing for chlorine is introduced when a representative sample is not obtained. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, then cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, the carefully fill to the 10-mL mark. Perform the chlorine analysis immediately.

Treating Analysis Labware

Glassware used in the test must be chlorine demand-free. Treat all glassware with a dilute solution of chlorine bleach prepared by adding 0.5 mL of commercial bleach to 1 liter of water. Soak glassware in this solution at least one hour. After soaking, rinse the glassware with large amounts of deionized water and allow to dry before use.

Treat the Pour/Flow-Thru Cell similarly with dilute bleach and let stand for several minutes and then rinse several times with deionized water.

Cleaning The Pour-Thru Cell

The Pour/Flow-Thru Cell may accumulate a buildup of colored reaction products, especially if the reacted solutions are allowed to stand in the cell for long periods after measurements. Remove the buildup by rinsing the cell with 5.25 N sulfuric acid followed by several rinsings with deionized water.

Interferences

Oxidized manganese reacts directly with DPD. The apparent chlorine result is about 3 µg/L per µg/L Mn^{7+}.

Mn^{2+} does not interfere up to 5000 µg/L.

Bromine, iodine, ozone, and other strong oxidizing agents may also interfere.

Copper and iron do not interfere up to 1000 µg/L each.*

Nitrite interference will vary according to the following table:

mg/L Nitrite*	Apparent µg/L Chlorine
2.0	3
5.0	5
10.0	7
15.0	16
20.0	18

* Monitor nitrite with Hach Water Quality Nitrate/Nitrite Test Strips (Cat. No. 27454-25). Dilution may be necessary for higher nitrite levels.

Summary of Method

Several modifications to the normal DPD chlorine method are necessary to measure trace levels of chlorine in treated wastewaters. The 1-inch Pour-Thru or Flow-Thru Cell **must** be used in the spectrophotometer. Liquid reagents are also **required**. The reproducible optics of the Pour/Flow-Thru Cell give more consistent readings than is possible with movable sample cells, resulting in more stable readings.

The reagents are packaged in ampules and sealed under argon gas to ensure stability. Use of liquid reagents eliminates any slight turbidity that might be caused by using powdered reagents. Due to the possible oxidation of the reagents (which could give a positive chlorine reading in the blank), a reagent blank must be determined at least once per day for each lot of reagents used. This reagent blank is subtracted from the sample result and the corrected value is the actual chlorine concentration.

It is essential that interfering sample turbidity be removed using a 3-micron membrane filter. To avoid loss of chlorine, the filtration is performed **after** reaction of DPD with chlorine in the sample. The filter used in the test has been specifically selected as to not retain the colored reaction product. Sample color is compensated by zeroing the spectrophotometer on a filtered sample.

* Measure copper with Hach Water Quality Copper Test Strips (Cat. No. 27451-25) and iron with Hach Water Quality Iron Test Strips (Cat. No.27453-25).

REQUIRED REAGENTS

Description	Cat. No.
ULR Chlorine Reagent Set (about 20 tests)	25630-00
Includes: (1) 24930-23, (1) 24931-20, (1) 24932-20	
ULR Chlorine Filter Apparatus Set	25956-00

Description	Quantity Required per test	Unit	Cat. No.
Blanking Reagent Solution	1 mL	29 mL	24930-23
DPD Indicator Solution	1 mL	20/pkg	24932-20
ULR Chlorine Buffer	1 mL	20/pkg	24931-20

REQUIRED APPARATUS

Beaker, 250 mL	1	each	500-46
Cylinder, mixing, graduated, 50 mL	1	each	1896-41
Flow-Thru Cell Assembly Kit (DR/4000)	1	each	48070-04
OR			
Pour-Thru Cell Assembly Kit (DR/2010)	1	each	45215-00
Pipet Tips, for TenSette® Pipet	2	50/pkg	21856-96
TenSette® Pipet, 0.1 to 1.0 mL	1	each	19700-01

OPTIONAL REAGENTS

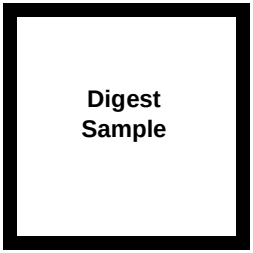
Chlorine Standard Sol'n, 2-mL Voluette™ Ampules, 20-30 mg/L	20/pkg	26300-20
Sulfuric Acid Solution, 5.25N	1L	2449-53
Water, deionized	4L	272-56
Water Quality Test Strip, total copper	25/pkg	27451-25
Water Quality Test Strip, total iron	25/pkg	27453-25
Water Quality Test Strip, nitrate and nitrite	25/pkg	27454-25

OPTIONAL APPARATUS

Ampule Breaker	1		24846-00
Bottle, wash, 250 mL		each	620-31
Membrane Filters, 3-micron	25/pkg		25940-25

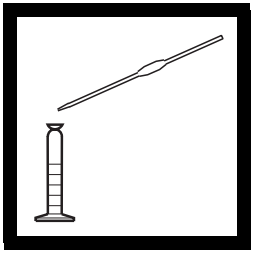
CHROMIUM, TOTAL

Alkaline Hypobromite Oxidation Method* **
Range: Liquids- 0.04–3000 mg/L; Solids- 5–30000 mg/kg

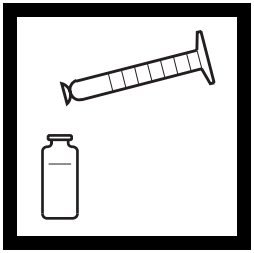


1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

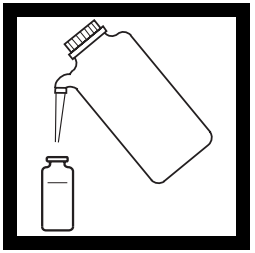
Note: *This method is USEPA approved only if preceded by an EPA-approved nitric acid digestion. The Digesdahl digestion is not EPA-approved and cannot be used for reporting.*



2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 25-mL mixing cylinder. If the aliquot is more than 0.5 mL, adjust the pH according to the instruction following the digestion method.

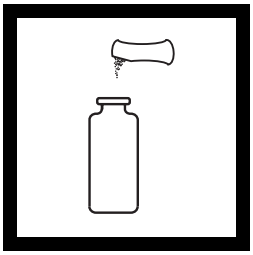


3. Fill a sample cell with 25 mL of the diluted sample from *step 2*.

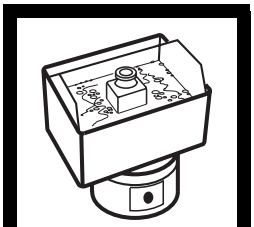


4. Fill a second 25-mL sample cell with the diluted sample (the blank)

Note: *For more accurate results, use a deionized water blank and treat it the same as the sample in steps 5-14. Subtract the results from the final reading.*

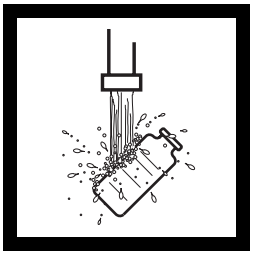


5. Add the contents of one Chromium 1 reagent Powder Pillow to one sample cell (the prepared sample). Swirl to mix.



6. Place the prepared sample into a boiling water bath.

7. Begin a five-minute reaction period.

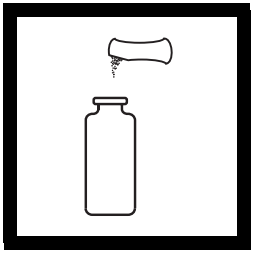


8. When the beeper sounds, remove the prepared sample. Using running tap water, cool the cell to 25 °C.

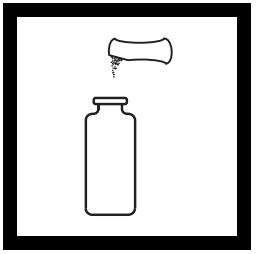
Note: *Use finger cots to handle the hot sample cell.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Procedure is equivalent to Standard Method 3500-CR D for wastewater.

9. Add the contents of one Chromium 2 Reagent Powder Pillow to the cell Swirl to mix.



10. Add the contents of one Acid Reagent Powder Pillow to the cell. Swirl to mix.



11. Add the contents of one ChromaVer 3 Chromium Reagent Powder Pillow. Swirl to mix.

Note: *A purple color will form if chromium is present.*

Note: *ChromaVer 3 is white to tan in color. Replace brown or green powder. Undissolved powder does not affect accuracy.*

12. Wait five minutes for color development

Note: *For turbid samples, treat the blank as described in Steps 5 through 10.*

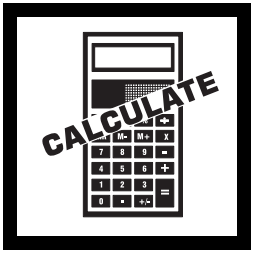
13. Place the blank into the cell holder. Zero the instrument using the settings below.

DR/800s
Program No. 13

DR/2010
Program No. 100
540 nm

DR/4000
Program No.1560
540 nm

14. Place the sample in the cell holder. Read the mg/L chromium.



15. Use the equation below the Total Chromium Sample and Analysis Volume Tables to calculate the true chromium concentration

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Cr Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.04-1.8	40.0	20.0	25 mL
0.13-7.5	20.0	10.0	25 mL
0.50-30	10.0	5.00	25 mL
5.0-300	5.00	1.00	25 mL
50-3000	1.00	0.50	25 mL

Solids

Expected Cr Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
5-150	0.500	20.0	25 mL
13-375	0.400	10.0	25 mL
35-1000	0.300	5.00	25 mL
250-7500	0.200	1.00	25 mL
1000-30000	0.100	0.50	25 mL

Calculation For Final Concentration:

$$\frac{A \times 2500}{B \times C} = \text{mg/L or mg/kg total Cr}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-washed glass or plastic containers. To preserve samples, adjust the pH to 2 or lower with nitric acid (about 2 mL per liter). Store preserved samples at room temperature up to six months. Adjust the pH to about 4 with 5.0 N Sodium Hydroxide before analysis. Correct the test results for volume additions.

Accuracy Check

Standard Additions Method

a. Perform the chromium method and note the analysis volume used and the mg/L Cr of the sample in *step 15*.

b. Pipet the same analysis volume into three 25-mL graduated cylinders.

c. Snap the top off a Trivalent Chromium Voluette Ampule Standard, 12.5 mg/L as Cr^{3+}.

d. Use the TenSette pipet to add 0.1, 0.2, and 0.3 mL of standard to the three 25-mL mixing cylinders. Dilute to 25 mL if necessary. Mix each thoroughly.

e. Analyze each sample as described above. The chromium concentration should increase 0.05 mg/L for each 0.1 mL of standard added.

Standard Solution Method

Prepare a 0.25 mg/L trivalent chromium standard by diluting 5.00 mL of chromium standard solution, 50 mg/L as Cr^{3+}, to 1000 mL with deionized water. Prepare this solution daily. Perform the chromium procedure as described above. The mg/L Cr reading should be 0.25 mg/L.

Interferences

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.

Summary of Method

Trivalent chromium in the sample is oxidized to the hexavalent form by hypobromite ion under alkaline conditions. The sample is acidified. Total chromium content is determined by the 1,5-diphenylcarbohydrazide method.

REQUIRED REAGENTS

Description	Cat. No.
Total Chromium Reagent Set (100 Tests)	22425-00
Includes: (2) 2126-66, (1) 12066-99, (1) 2043-99, (1) 2044-99	

Description	Quantity Required Per Test	Unit	Cat. No.
Acid Reagent Powder Pillows	1 pillow	100/pkg	2126-99
ChromaVer® 3 Chromium Reagent Powder Pillows	1 pillow	100/pkg	12066-99
Chromium 1 Reagent Powder Pillows	1 pillow	100/pkg	2043-99
Chromium 2 Reagent Powder Pillows	1 pillow	100/pkg	2044-99
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Cylinder, mixing, graduated, 25 mL	1	each	20886-40
Hot plate, 3½-in. diameter, 120 Vac	1	each	12067-01
Hot plate, 3½-in. diameter, 240 Vac	1	each	12067-02
Water bath and rack	1	each	1955-55

Select one or more based on sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

OPTIONAL REAGENTS

Description	Unit	Cat. No.
Chromium, trivalent, standard solution, 50 mg/L Cr^{3+}	100 mL	14151-42
Chromium, trivalent, standard solution, Voluette™ ampule, 12.5 mg/L Cr^{3+}, 10 mL	16/pkg	14257-10
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution 1:1	500 mL	2540-49
Sodium Hydroxide Solution 5.0 N	50 mL* SCDB	2450-26

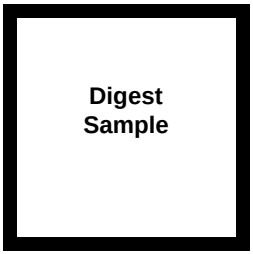
OPTIONAL APPARATUS

Description	Unit	Cat. No.
Ampule Breaker Kit	each	21968-00
Cylinder, graduated, polypropylene, 25 mL	each	1081-40
Cylinder, graduated, mixing, 25 mL	each	20886-40
Finger Cots	2/pkg	14647-02
Flask, volumetric, 1000 mL	each	547-53
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable	each	51700-10
Pipet, serological, 2 mL	each	532-36
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 5.00 mL	each	14515-37
Pipet Filler, safety bulb	each	14651-00

* Contact Hach for larger sizes.

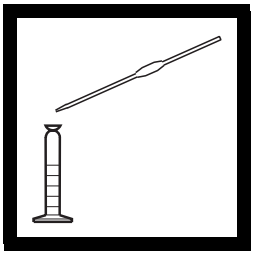
COBALT

PAN Method
Range: Liquids- 0.05–10,000 mg/L; Solids- 5–100,000 mg/kg



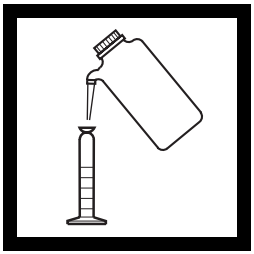
1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *SECTION 3*.

Note: *For best results, analyze a reagent blank for each lot of reagents. Subtract the blank value from the displayed sample value.*



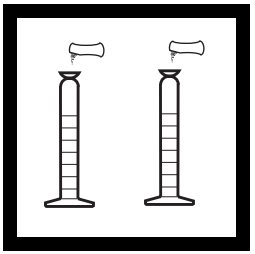
2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 10-mL mixing cylinder. If the aliquot is more than 0.2 mL, adjust the pH according to the instruction following the digestion method. Dilute to the 10-mL mark with deionized water.

Note: *For proof of accuracy, use a 0.5 mg/L cobalt standard in place of the sample (preparation given in Accuracy Check).*



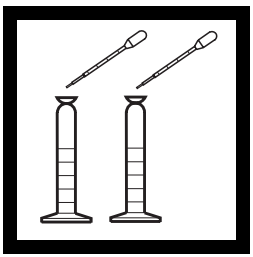
3. Fill another 10-mL cylinder with 10 mL of deionized water (the blank).

Note: *If sample is less than 10 °C (50 °F), warm to room temperature prior to analysis.*



4. Add the contents of one Phthalate-Phosphate Reagent Powder Pillow to each cylinder. Stopper each cylinder. Immediately shake to dissolve.

Note: *Invert the cylinder until all the powder dissolves. This is critical if the sample contains iron.*

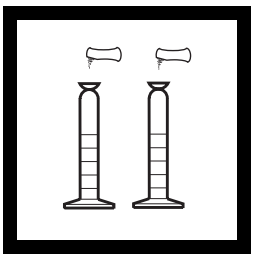


5. Add 0.5 mL of 0.3% PAN Indicator Solution to each cylinder. Stopper. Invert several times to mix.

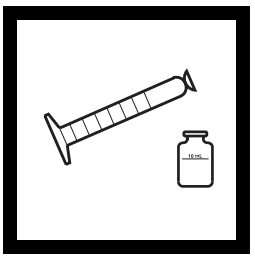
Note: *Use plastic dropper provided.*

6. Wait three minutes for color to develop.

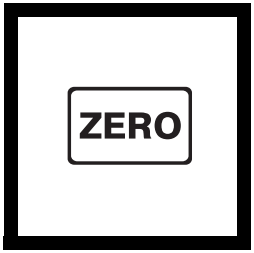
Note: *The sample solution color may vary from green to dark red, depending on the chemical makeup of the sample. The blank should be yellow.*



7. When the timer beeps, add the contents of one EDTA Reagent Powder Pillow to each cylinder. Stopper. Shake to dissolve.



8. Pour the contents of each cylinder into separate 10-mL sample cells.

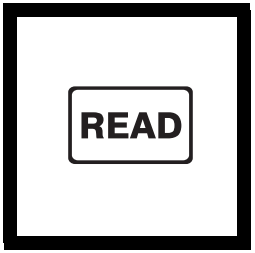


9. Place the blank into the cell holder. Close the light shield. Zero the instrument with the blank, using the settings below.

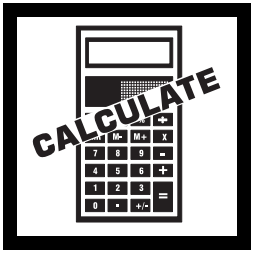
DR/800s
Program No. NA

DR/2010
Program No. 110
620 nm

DR/4000
Program No.1600
620 nm



10. Place the sample in the cell holder. Read the mg/L cobalt.



11. Use the equation below the Cobalt Sample and Analysis Volume Tables to calculate the true cobalt concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Co Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.05-6.25	40.0	8.00	10 mL
0.13-25	20.0	4.00	10 mL
0.5-100	10.0	2.00	10 mL
5-1000	5.00	0.40	10 mL
50-10000	1.00	0.20	10 mL

Solids

Expected Co Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
5-500	0.500	8.00	10 mL
75-1250	0.400	4.00	10 mL
35-3300	0.300	2.00	10 mL
250-25000	0.200	0.40	10 mL
1000-100000	0.100	0.20	10 mL

Calculation For Final Concentration:

$$\frac{A \times 1000}{B \times C} = \text{mg/L or mg/kg Total Co}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Preserved samples can be stored up to six months at room temperature. Be sure the sample pH is less than 8 or some loss of cobalt as a precipitate will occur. Correct test results for volume additions.

Accuracy Check

Standard Solution Method

Prepare the working stock solution daily by diluting 10.00 mL of cobalt Standard Solution, 1000 mg/L as Co, to 1000 mL with deionized water. This is a 10 mg/L working standard. Prepare a 1.0-mg/L cobalt standard solution by diluting 10.0 mL of a 10-mg/L working stock solution to 100 mL in a volumetric flask.

Interferences

The following may interfere in concentrations exceeding those listed below.

Al^{3+}	32 mg/L
Ca^{2+}	1000 mg/L as ($CaCO_3$)
Cd^{2+}	20 mg/L
Cl^-	8000 mg/L
Cr^{6+}	40 mg/L
Cu^{2+}	15 mg/L*
F^-	20 mg/L
Fe^{3+}	10 mg/L*
K^+	500 mg/L
Mg^{2+}	400 mg/L
Mn^{2+}	25 mg/L
Mo^{6+}	60 mg/L
Na^+	5000 mg/L
Pb^{2+}	20 mg/L
Zn^{2+}	30 mg/L

* Monitor iron with Water Quality Iron Test Strips (Cat. No. 27453-25) and copper with Water Quality Copper Test Strips (Cat. No. 27451-25).

Summary of Method

After buffering the sample and masking any Fe^{3+} with pyrophosphate, the cobalt is reacted with 1-(2-Pyridylazo)-2-Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal-PAN complexes except nickel and cobalt. Both nickel and cobalt can be determined on the same sample.

REQUIRED REAGENTS

Description	Cat. No.
Cobalt Reagent Set, 10 mL size (100 Tests)	26516-00
Includes: (2) 7005-99, (1) 21502-32, (2) 26151-49	

Description	Quantity Required Per Test	Unit	Cat. No.
EDTA Reagent Powder Pillows	2 pillows	100/pkg	7005-99
Phthalate-Phosphate Reagent Powder Pillows, 10-mL size	2 pillows	100/pkg	26151-99
PAN Indicator Solution, 0.3%	1.0 mL	100 mL	21502-32
Water, deionized	10 mL	4 L	272-56

REQUIRED APPARATUS

Cylinder, mixing, graduated, 10 mL	2	each	20886-38
Stopper, HDPE, #13	2	6/pkg	20955-13
Pipet, TenSette®, 0.1-1.0 mL	1	each	19700-01
Pipet Tips, for TenSette® Pipet	2	50/pkg	21856-96

Select one or more based an sample amount and analysis volume:

Pipet, volumetric, Class A, 8.0 mL	1	each	14515-08
Pipet, volumetric, Class A, 4.0 mL	1	each	14515-04
Pipet, volumetric, Class A, 2.0 mL	1	each	14515-36

OPTIONAL REAGENTS

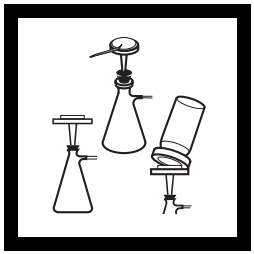
Cobalt Standard Solution, 1000 mg/L Co	100 mL		21503-42
Nitric Acid, ACS	500 mL		152-49
Nitric Acid Solution, 1:1	500 mL		2540-49

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Dropper, 0.5 and 1.0 mL mark	5/pkg	14197-05
Flask, volumetric, Class A, 100 mL	each	14574-42
Flask, volumetric, Class A, 1000 mL	each	14574-53
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable	each	51700-10
Pipet, serological, 1 mL	each	532-35
Pipet, serological, 5 mL	each	532-37
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 10.0 mL	each	14515-38
Pipet Filler, safety bulb	each	14651-00
Thermometer, -10 to 110 °C	each	1877-01
Water Quality Test Strips, total iron	25/pkg	27453-25
Water Quality Test Strips, total copper	25/pkg	27451-25

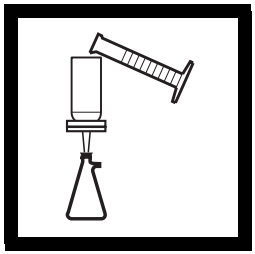
COLOR, TRUE AND APPARENT

APHA Platinum-Cobalt Standard Method* **
Range: 0 to 500 units

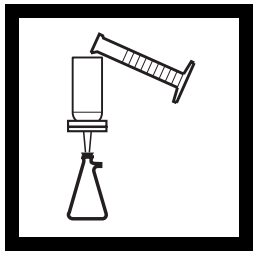


1. Assemble the filtering apparatus (membrane filter, filter holder, filter flask, and aspirator).

Note: *To test for apparent color, do not filter; begin at step 4 and skip Step 5.*



2. Rinse the filter by pouring about 50 mL of deionized water through the filter. Discard the rinse water.

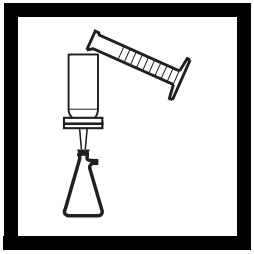


3. Pour another 50 mL of deionized water through the filter. Keep this for *step 4*.

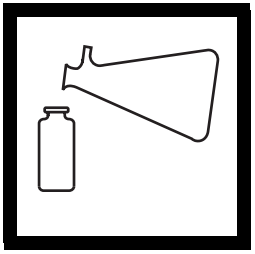
4. Fill a sample cell (the blank) with 25 mL of filtered deionized water. Discard the excess.

Note: *For apparent color use unfiltered deionized water.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater.* DR/4000 program 1680 is equivalent to NCASI method 253 for pulp and paper effluent (requires pH adjustment).
** *Wat. Re*s. Vol. 30, No 11, pp. 2771-2775, 1996.

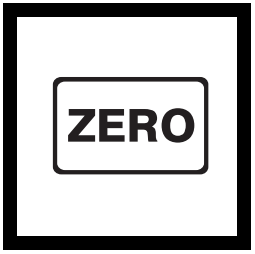


5. Pour about 50 mL of sample through the filter.



6. Fill a second sample cell (the prepared sample) with 25 mL of the filtered sample.

Note: *For apparent color, use unfiltered sample.*

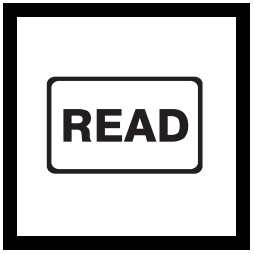


7. Place the blank into the cell holder. Zero the instrument with the blank using the settings below.

DR/800s
Program No. 19

DR/2010
Program No. 120
455 nm

DR/4000
Program No.1670
455 nm
or
Program No. 1680
465 nm



8. Place the sample in the cell holder. Read the Pt-Co units color.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Analyze the sample as soon as possible after collection for best results. If prompt analysis is impossible, fill bottles completely and cap tightly. Avoid excessive agitation or prolonged contact with air. Samples can be stored for 48 hours by cooling to 4 °C (39 °F). Warm to room temperature before running the test.

Accuracy Check

Standard Solution Method

A 500 Platinum-Cobalt Units Color Standard solution is available for checking test accuracy. A 250 Platinum-Cobalt Units Standard can be made by pipetting 50.0 mL of the 500 Platinum-Cobalt Units Standard into a 100-mL volumetric flask and diluting to volume with deionized water. A ready-to-use 15 Platinum-Cobalt Units Standard is also available. The Pour-Thru or Flow-Thru Cell is recommended when using the 15 Platinum-Cobalt Units Standard.

Summary of Method

Color may be expressed as apparent or true color. The apparent color includes color from dissolved materials plus that from suspended matter. By filtering or centrifuging out the suspended materials, the true color can be determined. The procedure describes true color analysis. If apparent color is desired, it can be determined by measuring an unfiltered water sample. The stored program is used for both forms of color.

For low-level color measurements, the Pour-Thru Cell or Flow-Thru Cell is recommended.

The stored program is calibrated in color units based on the APHA-recommended standard of 1 color unit being equal to 1 mg/L platinum as chloroplatinate ion.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Units	Cat. No.
Water, deionized	50 mL	4 L	272-56

REQUIRED APPARATUS

Aspirator, vacuum	1	each	2131-00
Filter Holder, 47 mm, 300 mL graduated	1	each	13529-00
Filter, membrane, 47 mm, 0.45 microns	1	100/pkg	13530-00
Flask, filtering, 500 mL	1	each	546-49
Stopper, No 7, one hole	1	6/pkg	2119-07
Tubing, Rubber	1	12 ft	560-19

OPTIONAL REAGENTS

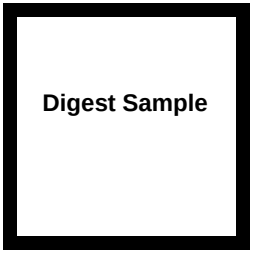
Color Standard Solution, 500 platinum-cobalt units		1 L	1414-53
Color Standard Solution, 15 platinum-cobalt units		1 L	26028-53

OPTIONAL APPARATUS

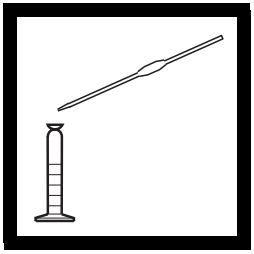
Cylinder, graduated, 50-mL, glass		each	508-41
Flask, volumetric, Class A, 100 mL		each	14574-42
Pipet, volumetric, Class A, 50 mL		each	14515-41
Thermometer, -10 to 110 °C		each	1877-01

COPPER, TOTAL

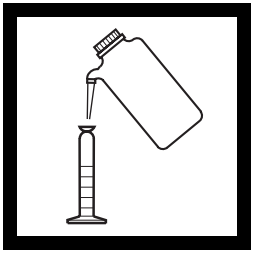
Bicinchoninate Method
Range: Liquids- 0.07–25000 mg/L; Solids- 6–250000 mg/kg



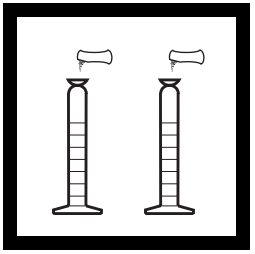
1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *SECTION 3*.



2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 25-mL mixing cylinder. If the aliquot is more than 0.5 mL, adjust the pH according to the instructions following the digestion method. Dilute to the 25-mL mark with deionized water.

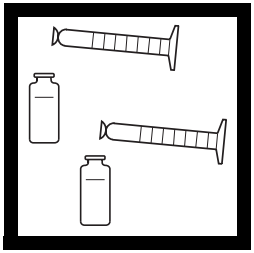


3. Fill a second 25-mL graduated mixing cylinder with 25 mL of deionized water.



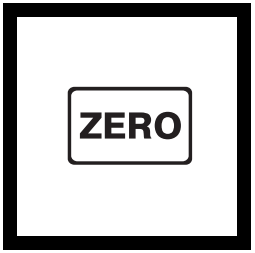
4. Add the contents of one CuVer 2 reagent Powder Pillow to each cylinder. Stopper and invert to mix.

Note: *If copper is present, the solution will turn purple.*



5. Pour the contents of each cylinder into separate 25-mL sample cells.

6. Wait at least two minutes for color to develop.



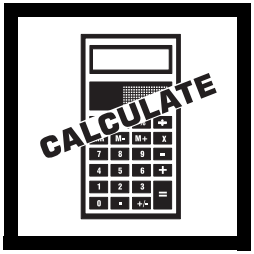
7. Place the blank into the cell holder. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 20

DR/2010
Program No. 135
560 nm

DR/4000
Program No. 1700
560 nm

8. Place the sample in the cell holder. Read the mg/L copper.



9. Use the equation below the Copper Sample and Analysis Volume Tables to calculate the true copper concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010, and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Cu Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.07-15	40.0	20.0	25 mL
0.25-60	20.0	10.0	25 mL
1-250	10.0	5.00	25 mL
10-2500	5.00	1.00	25 mL
100-25000	1.00	0.50	25 mL

Solids

Expected Cu Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
6-1250	0.500	20.0	25 mL
15-3125	0.400	10.0	25 mL
35-8300	0.300	5.00	25 mL
265-62500	0.200	1.00	25 mL
1100-250000	0.100	0.50	25 mL

Calculation For Final Concentration:

$$\frac{A \times 2500}{B \times C} = \text{mg/kg or mg/L Total Cu}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-cleaned glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Store preserved samples up to six months at room temperature. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method

a. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.

b. Snap the neck off a Copper PourRite Standard Ampule, 75 mg/L as Cu.

c. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard, respectively, to the mixing cylinders. Stopper and mix thoroughly.

d. Transfer the solution to 25-mL sample cells.

e. Analyze each sample as described in the procedure. The copper concentration should increase about 0.3 mg/L for each 0.1 mL of standard added.

Standard Solution Method

Prepare a 3.00 mg/L copper standard by pipetting 3.00 mL of Copper Standard Solution, 100 mg/L as Cu, into 100-mL volumetric flask. Dilute to volume with deionized water and mix well. Prepare this solution daily. Analyze this sample as described above. The copper concentration should be 3.00 mg/L.

Interferences

CuVer 2 Reagent is formulated to withstand high levels of calcium, iron, and aluminum without interference. It reacts directly with copper which is complexed by chelants such as EDTA.

If the sample is very acidic, adjust it to a pH greater than 4 before analysis. If a turbidity forms and turns black, silver interference is likely. This can be eliminated by adding 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, followed by filtration through a fine filter using the labware listed under *Optional Apparatus*. Use the filtered sample in the procedure.

Summary of Method

Copper in the sample reacts with a salt of bicinchoninic acid contained in CuVer 2 Copper Reagent to form a purple colored complex in proportion to the copper concentration.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
CuVer® 2 Copper Reagent Powder Pillows, 25-mL	1 pillow	100/pkg	21882-99
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Cylinder, graduated, mixing, 25 mL	2	each	20886-40
Pipet Filler, safety bulb	1	each	14651-00

Select one or more based on sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

OPTIONAL REAGENTS

Copper Standard Solution, 100 mg/L	100 mL	128-42
Copper Standard PourRite™ Ampule, 75 mg/L Cu, 2 mL	20/pkg	14247-20
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49

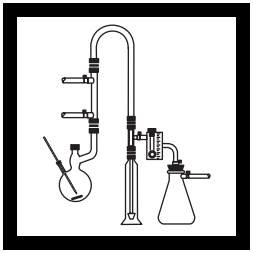
OPTIONAL APPARATUS

Flask, volumetric, 100 mL		each	547-42
Pipet, TenSette®, 0.1 to 1.0 mL		each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet		50/pkg	21856-96
Pipet, volumetric, Class A, 3.00 mL		each	14515-03

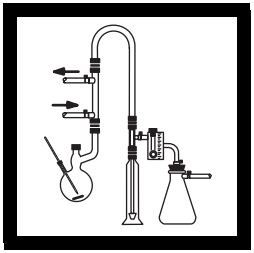
CYANIDE

Pyridine-Pyrazalone Method*
Range: 0.008–500 mg/L

Distillation

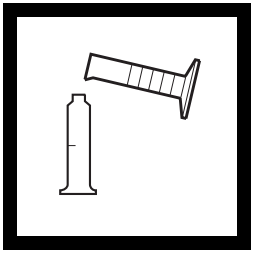


1. Set up the distillation apparatus for cyanide following the instructions in the *Distillation Apparatus Manual.* Do not connect the thistle tube yet. Place a stirring bar in the distillation flask.

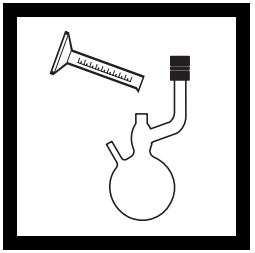


2. Turn on the water and make sure a steady flow is maintained through the condenser.

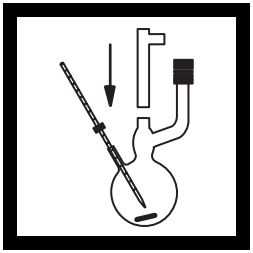
Note: *For proof of accuracy, use a 0.10-mg/L Cyanide standard Solution in place of the sample (see Accuracy check).*



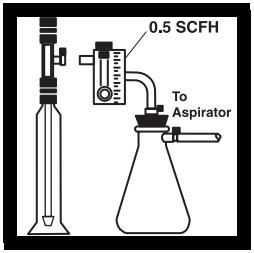
3. Remove and fill the distillation apparatus cylinder to the 50-mL mark with 0.25 N Sodium Hydroxide Standard Solution. Reassemble the cylinder and the distillation apparatus.



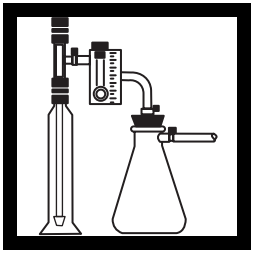
4. Fill a clean 250-mL graduated cylinder to the mark with sample and pour it into the distillation flask. For more concentrated samples, see *Table 1* on page *137*.



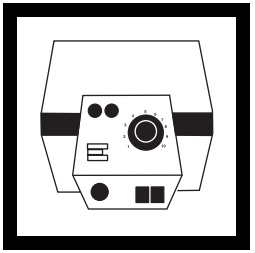
5. Attach the thistle tube to the flask.



6. Arrange the vacuum system as shown in the assembly drawing, but do not connect the vacuum tubing to the gas bubbler. Turn on the water to the aspirator full flow. Adjust the air flow meter to 0.5 SCFH (Standard Cubic Foot per Hour).

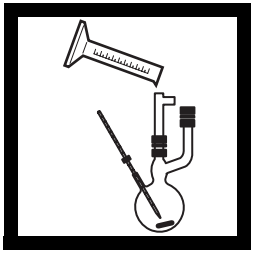


7. Connect the vacuum system tubing to the gas bubbler. Be sure the air flow is maintained (check flow meter) and that air is bubbling from the thistle tube and the gas bubbler.

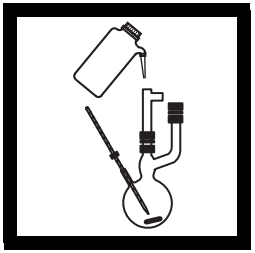


8. Turn the stirrer power switch and set the stir control to 5.

* Adapted from Epstein, Joseph, *Anal. Chem*. 19 (4), 272 (1947).

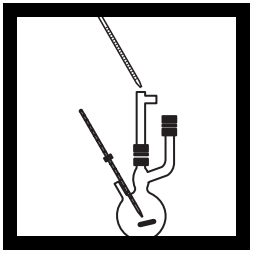


9. Using a 50-mL graduated cylinder, pour 50 mL of 19.2 N Sulfuric Acid Standard Solution into the distillation flask via the thistle tube.



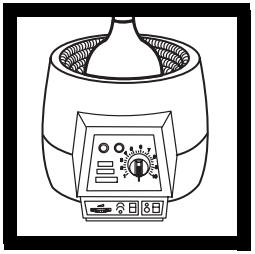
10. Using a water bottle, rinse the thistle tube walls with a small amount of deionized water.

11. Allow the solution to mix for three minutes.



12. Add 20 mL of Magnesium Chloride Reagent into the flask via the thistle tube. Rinse the thistle tube again.

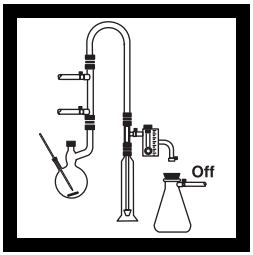
13. Allow the solution to mix for three more minutes.



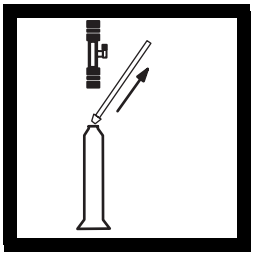
14. Verify there is a constant flow of water through the condenser, then turn the heater control to 10.

15. It is very important to monitor the distillation at this point. Once the sample boils, slowly lower the air flow to 0.3 SCFH. If the contents of the flask begin to back up through the thistle tube, increase the air flow until this no longer occurs. Allow the sample to boil for one hour.

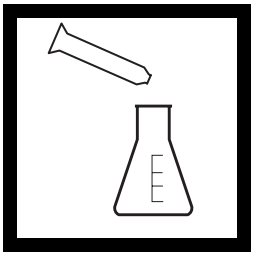
16. After one hour, turn the heat off, but maintain air flow for 15 minutes. Maintain the water flow through the condenser.



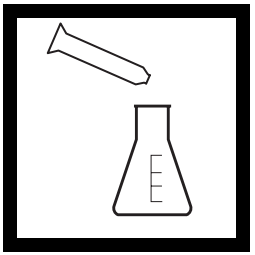
17. After 15 minutes, remove the rubber stopper on the 500-mL vacuum flask to break the vacuum. Turn off the water to the aspirator. Turn off water to the condenser.



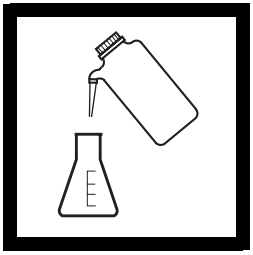
18. Remove the gas bubbler/cylinder assembly from the distillation apparatus. separate the gas bubbler from the cylinder.



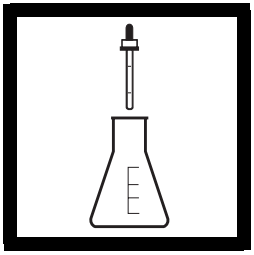
19. Pour the contents of the cylinder into a 250-mL Erlenmeyer flask.



20. Rinse the gas bubbler, cylinder, and J-tube connector with deionized water. Add the washings to the Erlenmeyer flask.

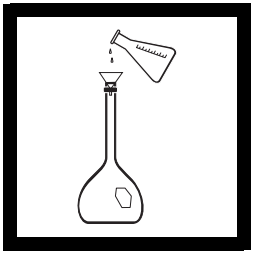


21. Fill the flask to the 200-mL mark with deionized water. Gently swirl to mix.



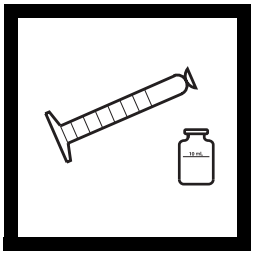
22. Add three drops of Phenolphthalein Indicator Solution. Adjust the pH of the sample with 2.5 N Hydrochloric Acid solution. Use a plastic dropper and add the Hydrochloric Acid Solution drop-wise until the solution becomes colorless.

Note: *Do not add sulfuric acid to neutralize the sample.*



23. Transfer the contents into a 250-mL volumetric flask. Dilute to volume with deionized water. The sample is ready for colorimetric analysis or measurement with a cyanide ion-selective electrode.

Colorimetric Analysis

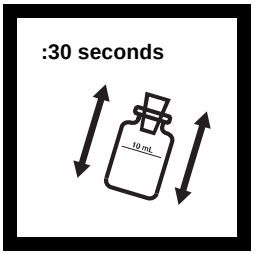


1. Using a graduated cylinder, pour 10 mL of sample into a 10-mL sample cell.

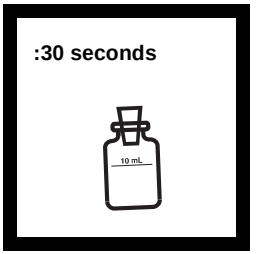
Note: *For best results, run a reagent blank using deionized water as the sample. Subtract the blank value from the displayed reading.*

2. Add the contents of one CyaniVer 3 Cyanide Reagent Powder Pillow. Stopper the sample cell.

Note: *The timing for steps 2-8 is critical. It may help to open the necessary reagents before starting.*

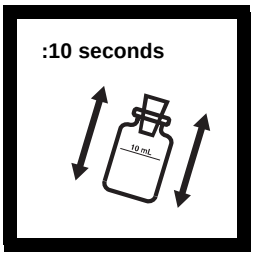


3. Shake the sample cell for 30 seconds.



4. Wait an additional 30 seconds while the sample cell is undisturbed.

5. Add the contents of one CyaniVer 4 Cyanide Reagent Powder Pillow. Stopper the sample cell.

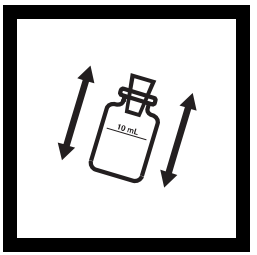


6. Shake the sample cell for 10 seconds. Immediately proceed with *step 7*.

Note: *Accuracy is not affected by undissolved CyaniVer 4 Cyanide Reagent Powder.*

7. Add the contents of one CyaniVer 5 Cyanide Reagent Powder Pillow. Stopper the cell.

Note: *Delaying the addition of the CyaniVer 5 Cyanide Reagent Powder for more than 30 seconds after the addition of the CyaniVer 4 Cyanide Reagent Powder will give lower test results.*

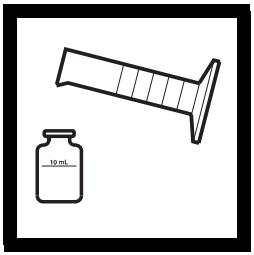


8. Shake vigorously to completely dissolve the CyaniVer 5 Cyanide Reagent Powder (the prepared sample).

Note: *If cyanide is present, a pink color will develop which then turns blue after a few minutes.*

9. Begin a 30-minute reaction period.

Note: *Samples at less than 25 °C require longer reaction time and samples at greater than 25 °C give low test results.*



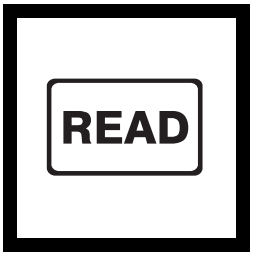
10. When the timer beeps, fill another 10-mL sample cell (the blank) with 10 mL of sample.

11. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 23

DR/2010
Program No. 160
612 nm

DR/4000
Program No.1750
612 nm



12. Place the sample in the cell holder. Read the mg/L cyanide.

Use the equation below the Cyanide Sample and Analysis Volume Table (below) to calculate the true cyanide concentration.

Table 1 Sample Dilution Volumes

Expected CN Concentration (mg/L)	Sample Volume (mL)	Dilute To
0.008–0.2	250	250
0.05–1	50	250
0.25–5	10	250
0.50–10	5	250
2.50–50	1	250
5–100	0.5	250
25–500	0.1	250

$$\frac{A \times 250}{B} = \text{mg/L Total CN}$$

A = mg/L reading from instrument

B = mL sample amount from table

Sampling and Storage

Samples collected in glass or plastic bottles should by analyzed as quickly as possible.

The presence of oxidizing agents, sulfides and fatty acids can cause cyanide loss during sample storage. Samples containing these substances must be pretreated as described in the following procedures before preservation with sodium hydroxide. If the sample contains sulfide and is not pretreated, it must be analyzed within 24 hours.

Preserve the sample by adding 4.0 mL of 5.0 N Sodium Hydroxide Standard Solution to each liter (or quart) of sample, using a glass serological pipet and pipet filler. Check the sample pH. Four mL of sodium hydroxide are usually enough to raise the pH of most water and wastewater samples to 12. Add more 5.0 N sodium hydroxide if necessary. Store the samples at 4 °C (39 °F) or less. Samples preserved in this manner can be stored for up to 14 days.

Before testing, samples preserved with 5.0 N sodium hydroxide or samples that are highly alkaline due to chlorination treatment processes or sample distillation procedures should be adjusted to approximately pH 7 with 2.5 N Hydrochloric Acid Standard Solution. Where significant amounts of preservative are used, a volume correction should be made.

Oxidizing Agents

Oxidizing agents such as chlorine decompose cyanides during storage. To test for their presence and eliminate their effect, pretreat samples as follows:

Note: *Measure chlorine with Hach Water Quality Total and Free Chlorine Test Strips (Cat. No. 27450-50).*

a. Take a 25-mL portion of the sample and add one drop of m-Nitrophenol Indicator Solution, 10 g/L. Swirl to mix.

b. Add 2.5 N Hydrochloric Acid Standard Solution dropwise until the color changes from yellow to colorless. Swirl the sample thoroughly after the addition of each drop.

c. Add two drops of Potassium Iodide Solution, 30 g/L, and two drops of Starch Indicator Solution, to the sample. Swirl to mix. The solution will turn blue if oxidizing agents are present.

d. If *step c* suggests the presence of oxidizing agents, add two level 1-g measuring spoonfuls of ascorbic acid per liter of sample.

e. Withdraw a 25-mL portion of sample treated with ascorbic acid and repeat *steps a* to *c*. If the sample turns blue, repeat *steps d* and *e*.

f. If the 25-mL sample remains colorless, adjust the remaining sample to pH 12 for storage with 5 N Sodium Hydroxide Standard Solution (usually 4 mL/L).

g. Perform the procedure given under Interferences, Reducing Agents, to eliminate the effect of excess ascorbic acid, before following the cyanide procedure.

Sulfides

Sulfides will quickly convert cyanide to thiocyanate (SCN). To test for the presence of sulfide and eliminate its effect, pretreat samples as follows:

a. Place a drop of sample on a disc of hydrogen sulfide test paper that has been wetted with pH 4 Buffer Solution.

b. If the test paper darkens, add a 1-g measuring spoon of lead acetate to the sample. Repeat *step a*.

c. If the test paper continues to turn dark, keep adding lead acetate until the sample tests negative for sulfide.

d. Filter the lead sulfide precipitate through filter paper and a funnel. Preserve the sample for storage with 5 N Sodium Hydroxide Standard Solution or neutralize to a pH of 7 for analysis.

Fatty Acids

Caution: Perform this operation in a hood as quickly as possible.

When distilled, fatty acids will pass over with cyanide and form soaps under the alkaline conditions of the absorber. If the presence of fatty acid is suspected, do not preserve samples with sodium hydroxide until the following pretreatment is performed. The effect of fatty acids can be minimized as follows:

a. Acidify 500 mL of sample to pH 6 or 7 with Acetic Acid Solution.

b. Pour the sample into a 1000-mL separatory funnel and add 50 mL of hexane.

c. Stopper the funnel and shake for one minute. Allow the layers to separate.

d. Drain off the sample (lower) layer into a 600-mL beaker. If the sample is to be stored, add 5 N Sodium Hydroxide Standard Solution to raise the pH to above 12.

Accuracy Check

Caution: Cyanides and their solutions, and the hydrogen cyanide liberated by acids, are very poisonous. Both the solutions and the gas can be absorbed through the skin.

Standard Additions Method

a. Prepare a 100 mg/L cyanide stock solution weekly by dissolving 0.1884 grams of sodium cyanide in deionized water and diluting to 1000 mL.

b. Pipet 5.0 mL of the 100 mg/L cyanide stock solution into a 100-mL volumetric flask to prepare a 5 mg/L cyanide working solution. Mix thoroughly.

c. Use a TenSette Pipet to add 0.1, 0.2, and 0.3 mL of the 5 mg/L solution to three 10-mL samples, respectively.

d. Swirl each sample and analyze according to the procedure. Each 0.1 mL of standard added should increase the cyanide concentration determined in Step 12 by 0.05 mg/L.

Standard Solution Method
Prepare a 100 mg/L cyanide stock solution weekly by dissolving 0.1884 grams of sodium cyanide in deionized water and diluting to 1000 mL.

Immediately before use, prepare a 0.10 mg/L cyanide working solution by diluting 1.00 mL of the 100 mg/L stock solution to 1000 mL using deionized water. Use this prepared standard in place of sample in *step 1*. Results should be 0.10 mg/L CN^-.

Interferences

Oxidizing and Reducing Agents
Large amounts of chlorine in the sample will cause a milky white precipitate after the addition of the CyaniVer 5 Reagent. If chlorine or other oxidizing agents are known to be present, or if reducing agents (such as sulfide or sulfur dioxide) are known to be present, pretreat the sample before testing as follows using adequate ventilation:

Oxidizing Agents

a. Adjust a 25-mL portion of the alkaline sample to between pH 7 and 9 with 2.5 N Hydrochloric Acid Standard Solution. Count the number of drops of acid added.

b. Add two drops of Potassium Iodide Solution and two drops of Starch Indicator Solution to the sample. Swirl to mix. The sample will turn blue if oxidizing agents are present.

c. Add Sodium Arsenite Solution drop-wise until the sample turns colorless. Swirl the sample thoroughly after each drop. Count the number of drops.

d. Take another 25-mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in Step a.

e. Subtract one drop from the amount of Sodium Arsenite Solution added in *step c*. Add this amount to the sample. Mix thoroughly.

f. Using 10 mL of this sample, continue with *step 1* of the cyanide procedure.

Reducing Agents

a. Adjust a 25-mL portion of the alkaline sample to between 7 and 9 with 2.5 N Hydrochloric Acid Standard Solution. Count the number of drops added.

b. Add four drops of Potassium Iodide Solution and four drops of Starch Indicator Solution to the sample. Swirl to mix. The sample should be colorless.

c. Add Bromine Water drop-wise until a blue color appears. Count the number of drops, and swirl the sample after the addition of each drop.

d. Take another 25 mL sample and add the total number of drops of Hydrochloric Acid Standard Solution counted in *step a*.

e. Add the total number of drops of Bromine Water counted in *step c* to the sample and mix thoroughly.

f. Using 10 mL of this sample, continue with *step 1* of the cyanide procedure.

Summary of Method

The pyridine-pyrazolone method used for measuring cyanide gives an intense blue color with free cyanide. A sample distillation is required to determine cyanide from transition and heavy metal cyanide complexes.

REQUIRED REAGENTS

Description	Cat. No.
Cyanide Reagent Set (100 Tests), 10-mL sample	24302-00
Includes: (1) 21068-69, (1) 21069-69, (1) 21070-69	

Description	Quantity Required Per Test	Unit	Cat. No.
CyaniVer® 3 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21068-69
CyaniVer® 4 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21069-69
CyaniVer® 5 Cyanide Reagent Powder Pillows	1 pillow	100/pkg	21070-69
Hydrochloric Acid Solution, 2.5 N	drops	100 mL MDB	1418-32
Magnesium Chloride Solution	20 mL	1 L	14762-53
Phenolphthalein Indicator Solution	drops	15 mL	1897-36
Sodium Hydroxide Standard Solution, 19.2 N	50 mL	500 mL	2038-49
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Bottle, wash, 500 mL	1	each	620-11
Cylinder, graduated, 25 mL	1	each	508-40
Cylinder, graduated, 50 mL	1	each	508-41
Cylinder, graduated, 250 mL	1	each	508-46
Distillation Apparatus, cyanide accessories	1	each	22658-00
Distillation Apparatus, general purpose accessories	1	each	22653-00
Distillation Apparatus Heater and Support Apparatus, 115 Vac, 60 Hz	1	each	22744-00
Distillation Apparatus Heater and Support Apparatus, 230 Vac, 50 Hz	1	each	22744-02
Flask, Erlenmeyer, 250 mL	1	each	505-46
Flask, volumetric, Class A, 250 mL	1	each	14574-46
Stoppers, rubber, solid, #1	1	12/pkg	2118-01

OPTIONAL REAGENTS

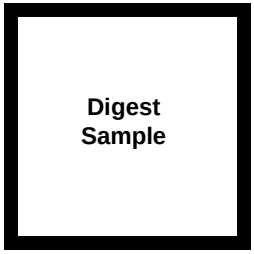
Acetic Acid Solution, 10%	500 mL	14816-49
Ascorbic Acid	100 g	6138-26
Bromine Water	29 mL	2211-20
Buffer Solution, pH 4.0	500 mL	12223-49
Hexanes, ACS	4 L	14478-17
HexaVer® Chelating Reagent Powder Pillows	100/pkg	243-99
Hydrochloric Acid Standard Solution, 2.5 N	100 mL MDB	1418-32
Lead Acetate, trihydrate, ACS	500 g	7071-34
Magnesium Chloride Solution	1000 mL	14762-53
m-Nitrophenol Indicator	100 mL MDB	2476-32
Potassium Iodide Solution, 30 g/L	100 mL MDB	343-32
Sodium Arsenite Solution, APHA	100 mL MDB	1047-32
Sodium Cyanide, ACS	28 g	184-20
Sodium Hydroxide Standard Solution, 0.25 N	1000 mL	14763-53
Sodium Hydroxide Standard Solution, 5.0 N	1 L	2450-53
Starch Indicator Solution	100 mL MDB	349-32
Sulfuric Acid Standard Solution, 19.2 N	500 mL	2038-49
Water Quality Test Strips, free and total chlorine	50/pkg	27450-50

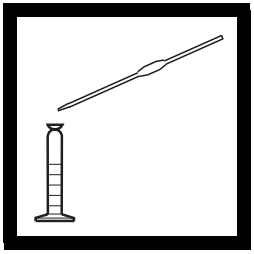
OPTIONAL APPARATUS

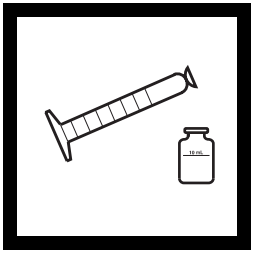
Description	Unit	Cat. No.
Balance, analytical, ScienTech, 115 V	each	26103-00
Balance, analytical, ScienTech, 230 V	each	26103-02
Beaker, glass, 600 mL	each	500-52
Cyanide Ion Selective Electrode Package	each	23486-00
Dropper, plastic	each	6080-00
Filter Paper, folded, 12.5 cm	100/pkg	1894-57
Flask, volumetric, 1000 mL	each	547-53
Flask, volumetric, Class A, 250 mL	each	14574-46
Funnel, poly, 65 mm	each	1083-67
Funnel, separatory, 500 mL	each	520-49
Hydrogen Sulfide Test Papers	100/pkg	25377-33
Midi-Distillation Apparatus, 4-port	each	26384-00
Midi-Distillation Apparatus, 10-port	each	26385-00
pH/ISE meter, laboratory, *sens**ion***™*4*	each	51775-10
Pipet Filler, safety bulb	each	14651-00
Scoop, double ended	each	12257-00
Spoon, measuring, 1.0 g	each	510-00
Support Ring, 10 cm (4 in.)	each	580-01
Support Stand	each	563-00
Timer, 3-channel	each	23480-00

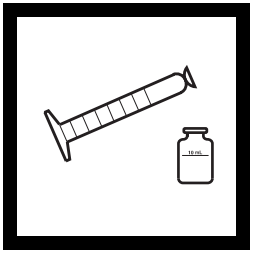
IRON, TOTAL

FerroVer Method (USEPA approved for reporting wastewater analyses)*
Range: Liquids- 0.1–15000 mg/L; Solids- 7.5–150,000 mg/kg









1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

Note: *If sample cannot be analyzed shortly after collection, see Sampling and Storage following these steps.*

Note: *This is an EPA-approved method only if preceded by an EPA-approved nitric acid digestion. The Digesdahl digestion procedure is not EPA approved and cannot be used for permit reporting purposes. See 3.1.2 on page 20 for the approved digestion.*

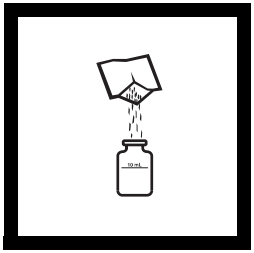
2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 25-mL mixing cylinder. If the aliquot is more than 0.5 mL, adjust the pH according to the instruction following the digestion method. Dilute to the 25-mL mark with deionized water.

3. Pour 10 mL of the sample into a 10-mL sample cell.

Note: *For more accurate results, run a reagent blank and subtract that value from the displayed reading.*

4. Fill a second 10-mL sample cell with 10 mL of sample (the blank).

* *Federal Register*, 45 (126) 43459 (June 27, 1980). See *step 1* note. Adapted from *Standard Methods for the Examination of Water and Wastewater.*

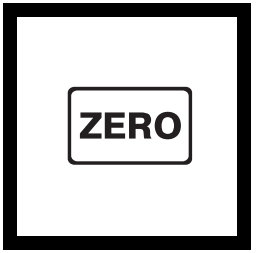


5. Add the contents of one FerroVer Iron reagent Powder Pillow to one of the sample cells (the prepared sample). Swirl to mix.

Note: *The solution will turn orange if iron is present.*

Note: *Accuracy is not affected by undissolved powder.*

6. Begin a three-minute reaction period.

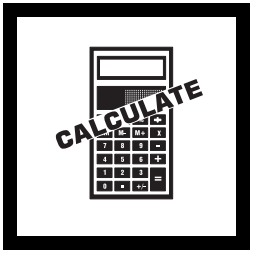


7. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 33

DR/2010
Program No. 265
510 nm

DR/4000
Program No.2165
510 nm



8. Place the sample in the cell holder. Read the mg/L iron.

Use the equation below the FerroVer Iron Sample and Analysis Volume Table (next page) to calculate the true iron concentration.

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Fe Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.1-9	40.0	20.0	25 mL
0.4-37.5	20.0	10.0	25 mL
1.5-150	10.0	5.00	25 mL
15-1500	5.00	1.00	25 mL
150-15000	1.00	0.50	25 mL

Solids

Expected Fe Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
7.5-750	0.500	20.0	25 mL
20-1875	0.400	10.0	25 mL
50-5000	0.300	5.00	25 mL
375-37500	0.200	1.00	25 mL
1500-150000	0.100	0.50	25 mL

Calculation For Final Concentration:

$$\frac{A \times 2500}{B \times C} = \text{mg/L or mg/kg Total Fe}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-cleaned glass or plastic containers. No acid addition is necessary if analyzing the sample immediately. To preserve samples, adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature.Correct the test result for volume additions.

Accuracy Check

Standard Additions Method

a. Perform the iron analysis and note the analysis volume used and the mg/L Fe of the sample.

b. Pipet the same analysis volume into three 25-mL graduated mixing cylinders.

c. Snap the neck off a 50 mg/L Iron Voluette Ampule Standard Solution.

d. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard, respectively, to the three mixing cylinders and mix thoroughly.

e. Transfer only 10 mL of solution to the 10-mL sample cells.

f. Analyze each standard addition sample as described above. The iron concentration should increase 0.2 mg/L for each 0.1 mL of standard added. If these increases do not occur, an interference is likely.

Standard Solution Method
Prepare a 1.00 mg/L iron standard by diluting 1.00 mL of Iron Standard Solution, 100 mg/L Fe, to 100 mL with deionized water. Or dilute 1.00 mL of an Iron Voluette Ampule Standard Solution (50 mg/L) to 50 mL in a volumetric flask. Prepare this solution daily.

Run the test, following the procedure above, using the 1.00 mg/L Iron Standard Solution. Results should be between 0.90 mg/L and 1.10 mg/L Fe.

Interferences

The following will not interfere below the levels shown:

Chloride	185,000 mg/L
Calcium	10,000 mg/L as $CaCO_3$
Magnesium	100,000 mg/L as $CaCO_3$
Molybdate Molybdenum	50 mg/L as Mo

A large excess of iron will inhibit color development. A diluted sample should be tested if there is any doubt about the validity of a result.

FerroVer Iron Reagent contains a masking agent which eliminates potential interferences from copper.

Summary of Method

FerroVer Iron Reagent reacts with all soluble iron and most insoluble forms of iron in the sample, to produce soluble ferrous iron. This reacts with the 1,10-phenanthroline indicator in the reagent to form an orange color in proportion to the iron concentration.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat No.
FerroVer® Iron Reagent Powder Pillows, 10 mL samples	1 pillow	100/pkg	21057-69
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Cylinder, graduated, mixing, 25 mL	2	each	20886-40

Select one or more based an sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

OPTIONAL REAGENTS

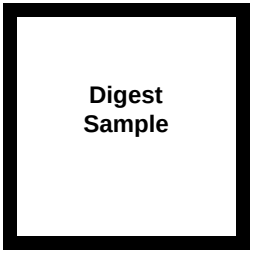
Ammonium Hydroxide, ACS	500 mL	106-49
Hydrochloric Acid Standard Solution, 6 N	500 mL	884-49
Hydrochloric Acid, ACS	500 mL	134-49
Iron Standard Solution, 100 mg/L	100 mL	14175-42
Iron Voluette™ Ampule Standard, 50 mg/L	16/pkg	14254-10
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
RoVer® Rust Remover	454 g	300-01
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Cylinder, graduated, glass, 25 mL	each	508-40
Cylinder, graduated, poly, 25 mL	each	1081-40
Cylinder, graduated, poly, 100 mL	each	1081-42
Filter Holder, membrane	each	2340-00
Filter Pump	each	2131-00
Flask, Erlenmeyer, 250 mL	each	505-46
Flask, filtering, 500 mL	each	546-49
Flask, volumetric, Class A, 50 mL	each	14574-41
Flask, volumetric, Class A, 100 mL	each	14574-42
Hot Plate, 3½ in.diameter, 120 Vac	each	12067-01
Hot Plate, 3½ in.diameter, 240 Vac	each	12067-02
pH Meter, *sens**ion***™*1*, portable	each	51700-10
pH Indicator Paper, 1 to 11 pH	each	391-33
Pipet Filler, safety bulb	each	14651-00
Pipet, serological, 2 mL	each	532-36
Pipet, serological, 5 mL	each	532-37
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00 mL	each	14515-35
Spoon, measuring, 0.1 g	each	511-00

IRON, TOTAL

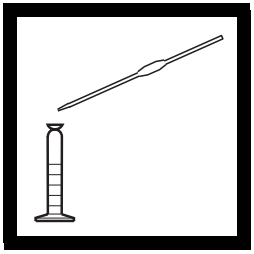
Ferrozine Method*
Range: Liquids- 0.04–6500 mg/L; Solids- 4–65000 mg/kg



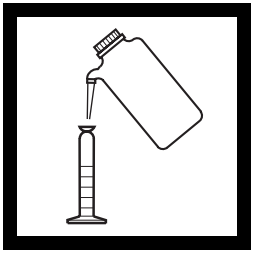
1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

Note: *If sample cannot be analyzed immediately after collection, see Sampling and Storage following these steps.*

Note: *Rinse glassware with 1:1 hydrochloric acid and deionized water before use to avoid errors due to iron deposits on the glass.*

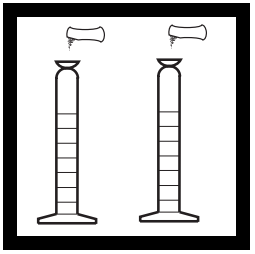


2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 25-mL mixing cylinder. If the aliquot is more than 0.5 mL, adjust the pH according to the instruction following the digestion method. Dilute to the 25-ml mark with deionized water.



3. Fill a second 25-mL graduated mixing cylinder with deionized water (the blank).

Note: *For proof of accuracy, a 0.4 mg/L iron standard solution (preparation given in the Accuracy Check) can be used in place of the sample.*



4. Add the contents of one FerroZine Iron Reagent Solution Pillow to each cylinder. Swirl to mix.

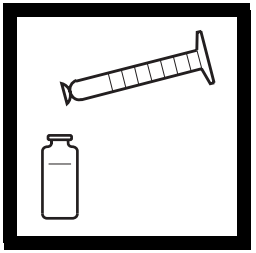
Note: *0.5 mL of FerroZine Iron Reagent Solution can be used in place of the solution pillow if preferred.*

Note: *If the sample contains rust, see Interferences following these steps.*

* Adapted from Stookey, L.L., *Anal. Chem*., 42 (7) 779 (1970)

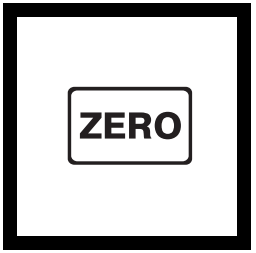
5. Begin a five-minute reaction period.

Note: *The solution will turn purple if iron is present.*



6. Pour the contents of each cylinder into separate 25-ml sample cells.

Note: *The Pour/Flow-Thru cell can be used.*

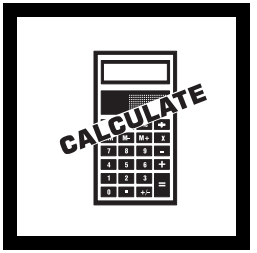


7. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 37

DR/2010
Program No. 260
562 nm

DR/4000
Program No.2175
562 nm



8. Place the sample in the cell holder. Read the mg/L iron.

Use the equation below the Ferrozine Iron Sample and Analysis Volume Tables to calculate the true iron concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Fe Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.04-4	40.0	20.0	25 mL
0.14-16	20.0	10.0	25 mL
0.55-65	10.0	5.00	25 mL
5.5-650	5.00	1.00	25 mL
55-6500	1.00	0.50	25 mL

Solids

Expected Fe Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
4-325	0.500	20.0	25 mL
7-810	0.400	10.0	25 mL
20-2150	0.300	5.00	25 mL
140-16250	0.200	1.00	25 mL
550-65000	0.100	0.50	25 mL

Calculation For Final Concentration:

$$\frac{A \times 2500}{B \times C} = \text{mg/L or mg/kg Total Fe}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with nitric acid (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature.

Accuracy Check

Standard Additions Method

a. Perform the FerroZine iron method and note the analysis volume used and the mg/L Fe in *step 8*.

b. Pipet the same analysis volume into three 25-mL graduated mixing cylinders.

c. Snap the neck off an Iron Voluette Ampule Standard, 25 mg/L Fe.

d. Use the TenSette pipet to add 0.1 mL 0.2 mL, and 0.3 mL of standard to the three cylinders.

e. Perform the Ferrozine iron method beginning with *step 3*.

f. The iron concentration should increase 0.1, 0.2 and 0.3 mg/L, respectively, compared to the concentration in *step 8*. If these increase do not occur, an interference is likely.

Standard Solution Method
Prepare a 0.4 mg/L iron working solution as follows:

a. Pipet 1.00 mL of iron standard solution, 100 mg/L Fe, into a 250 mL volumetric flask.

b. Dilute to volume with deionized water. This solution should be prepared daily. Analyze the working solution according to the above procedure.

Interferences

Copper and cobalt may interfere to give slightly high results.

If rust or hydroxides are present, boil the sample, with the FerroZine Iron Reagent added in Step 4, for one minute in a boiling water bath then cool to 24 °C (75 °F) before proceeding with *step 5*. The reduced sample volume should be returned to 25 mL with deionized water.

If the sample contains magnetite (black iron oxide) or ferrites, perform the following procedure.

a. Fill a 25-mL graduated cylinder with 25 mL of sample.

Note: *Do not allow to boil dry.*

b. Transfer the sample water into a 125-mL Erlenmeyer flask.

c. Add the contents of one FerroZine Iron Reagent Solution Pillow and swirl to mix.

d. Place the flask on a hot plate or over a flame and bring to a boil.

Note: *A purple color will develop if iron is present.*

e. Continue boiling gently for 20 to 30 minutes.

f. Return the boiled sample to the graduated cylinder. Rinse the Erlenmeyer flask with small amounts of deionized water and empty into the graduated cylinder.

g. Return the sample volume to the 25-mL mark with deionized water.

h. Pour the solution into a sample cell and swirl to mix.

i. Proceed with *steps 5* through *8*.

Summary of Method

The FerroZine Iron Reagent forms a purple colored complex with trace amounts of iron in samples that are buffered to a pH of 3.5. This method is applicable for determining trace levels of iron in chemical reagents and glycols.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
FerroZine® Iron Reagent Solution Pillows	1 pillow	50/pkg	2301-66
Water, deionized	25 mL	4 L	272-56

REQUIRED APPARATUS

Cylinder, mixing, graduated, 25-mL	2	each	20886-40

Select one or more based an sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

OPTIONAL REAGENTS

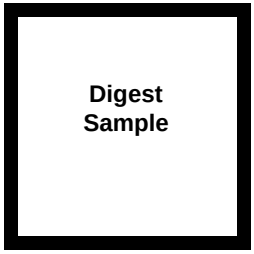
Hydrochloric Acid Solution, 1:1 (6N)	500 mL	884-49
FerroZine® Iron Reagent Solution	1000 mL	2301-53
Iron Standard Solution, 100 mg/L Fe	100 mL	14175-42
Iron Standard Solution, Voluette™ Ampule, 25 mg/L Fe, 10 mL	16/pkg	14253-10
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49

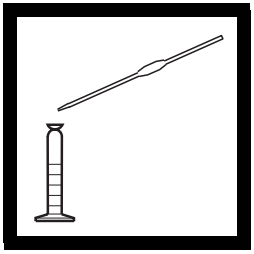
OPTIONAL APPARATUS

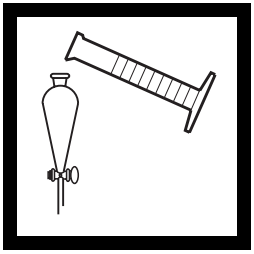
Cylinder, graduated, 25 mL	each	508-40
Dropper, calibrated, 0.5-mL & 1.0-mL mark	6/pkg	23185-06
Flask, Erlenmeyer, 125 mL	each	505-43
Flask, volumetric, 250 mL, Class A	each	14574-46
pH Meter, *sens**ion**™1*, portable	each	51700-10
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Thermometer, -10 to 110 °C	each	1877-01

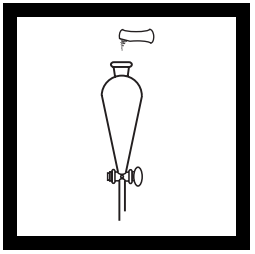
LEAD

Dithizone Method* (USEPA accepted for reporting wastewater analysis)**
Range: Liquids- 0.1–8000 mg/L; Solids- 8–80000 mg/kg









1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

Note: *If the sample cannot be analyzed immediately after collection, see Sampling and Storage following these steps.*

Note: *This method is USEPA-accepted only if preceded by an EPA-approved digestion. The Digesdahl digestion procedure is not EPA-approved and cannot be used for reporting purposes.*

2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 250-mL graduated cylinder. If the aliquot is more than 0.5 mL, adjust the pH according to the instructions following the digestion method. Dilute to the 250-mL mark with deionized water.

Note: *Clean all glassware with a 1:1 Nitric Acid Solution. Rinse with deionized water.*

3. Transfer the sample into a 500-mL separatory funnel.

Note: *Perform the procedure with proper ventilation or in a fume hood.*

4. Add the contents of one Buffer Powder Pillow, citrate type for heavy metals. Stopper the funnel. Shake to dissolve.

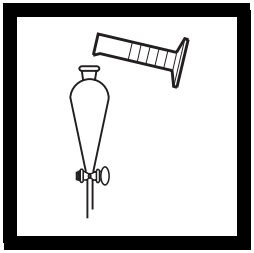
Note: *Spilled reagent will affect test accuracy and is hazardous.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater*
** Procedure is equivalent to Standard Method 3500-Pb D for wastewater. USEPA approved digestion required.

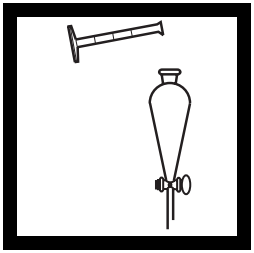
5. Add 50 mL of chloroform to a 50-mL graduated cylinder. Add the contents of one DithiVer Metals Reagent Powder Pillow. Stopper. Invert repeatedly to mix (DithiVer solution). Pour 30 mL of the DithiVer solution into a second 50-mL graduated cylinder.

Note: *Use adequate ventilation. The DithiVer Powder will not all dissolve. See DithiVer Solution Preparation.*

Note: *Most sludge samples will require a pretreatment extraction to remove copper and other interfering ions. See Interferences following these steps.*

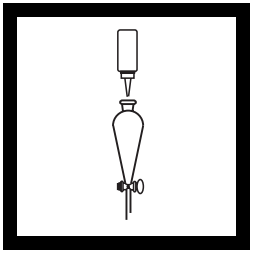


6. Add the 30 mL of DithiVer solution from the cylinder to the separatory funnel. Stopper. Invert. Open stopcock to vent.



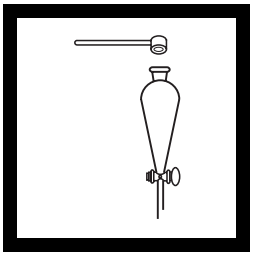
7. Add 5 mL of 5.0 N Sodium Hydroxide Standard Solution. Stopper. Invert. Open stopcock to vent. Shake the funnel once or twice and vent again.

Note: *Add a few drops of 5.25 N Sulfuric Acid Standard Solution if the solution turns orange on shaking. The blue-green color will reappear. To avoid high blanks, repeat procedure on new sample and use less sodium hydroxide.*



8. Continue adding 5.0 N Sodium Hydroxide Standard Solution dropwise until the color of the solution being shaken changes from blue-green to orange. Then add 5 more drops of 5.0 N Sodium Hydroxide Standard Solution.

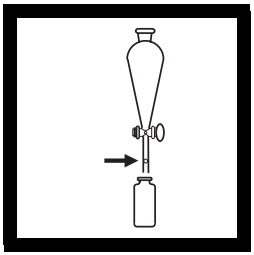
Note: *For most accurate results, adjust the sample to pH 11.0 to 11.5 using a pH meter, omitting the five additional drops of Sodium Hydroxide Standard Solution.*



9. Add two heaping 1.0- g scoops of potassium cyanide to the funnel. Stopper. Shake vigorously until the potassium cyanide is all dissolved (about 15 seconds).

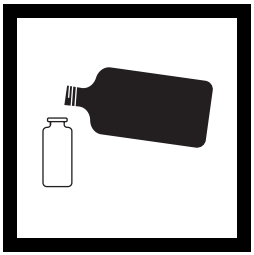
Note: *Wait one minute for the layers to separate. The bottom (chloroform) layer will be pink if lead is present.*

Note: *Potassium cyanide is a deadly poison. Do not allow ingestion, inhalating or contact with skin. Do not allow contact with acids or hydrogen cyanide gas may result.*



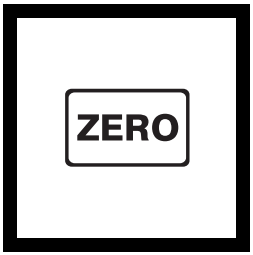
10. Insert a cotton plug the size of a pea into the delivery tube of the funnel and slowly drain the bottom (chloroform) layer into a dry 25-mL sample cell. Stopper. This is the prepared sample.

Note: *The lead-dithizone complex is stable for hours if the sample cell is kept tightly capped and out of direct sunlight.*



11. Fill a 25-mL sample cell (the blank) with chloroform. Stopper.

Note: *For more accurate results, run a reagent blank for each new lot of chloroform and DithiVer pillows. Use Hach deionized water in place of the sample. Subtract the blank value from the results. Hach chloroform is lead-free; chloroform from other sources may contain lead.*

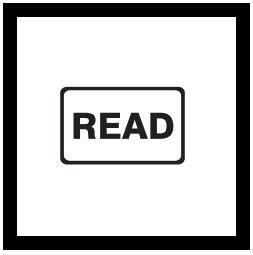


12. Zero the instrument with the blank, using the settings below.

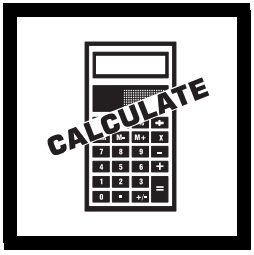
DR/800s
Program No. NA

DR/2010
Program No. 280
515 nm

DR/4000
Program No.2200
515 nm



13. Place the sample in the cell holder. Read the µg/L lead.



14. Use the equation below the Dithizone Lead Sample and Analysis Volume Tables to calculate the true lead concentration.

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Pb Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.1-5.0	40.0	20.0	250 mL
0.4-20	20.0	10.0	250 mL
1.5-80	10.0	5.00	250 mL
15-800	5.00	1.00	250 mL
150-8000	1.00	0.50	250 mL

Solids

Expected Pb Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
8-400	0.500	20.0	250 mL
20-1000	0.400	10.0	250 mL
50-2600	0.300	5.00	250 mL
400-20000	0.200	1.00	250 mL
1500-80000	0.100	0.50	250 mL

Calculation For Final Concentration:

$$\frac{A \times 25}{B \times C} = \text{mg/L or mg/kg Total Pb}$$

OR

$$\frac{A \times 25000}{B \times C} = \text{µg/L or µg/kg Total Pb}$$

A = µg/Lreading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Dithiver Solution Preparation, Storage And Blank

Store DithiVer Powder Pillows away from light and heat. A convenient way to prepare this solution is to add the contents of 10 DithiVer Metals Reagent Powder Pillows to a 500-mL bottle of chloroform. Invert several times until well mixed (carrier powder may not dissolve). Store dithizone solution in an amber glass bottle. This solution is stable for 24 hours.

A reagent blank on deionized water should be carried through the entire method to obtain the most accurate results. The amount of reagent blank determined on each lot of DithiVer Metals Reagent Powder Pillow is then subtracted from each reading obtained in *step 13*.

Sampling and Storage

Collect samples in acid cleaned glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples can be stored up to six months at room temperature. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method

a. Perform the lead method and note the analysis volume used and the µg/L Pb of the sample in *step 13.*

b. Pipet the same analysis volume into three 250-mL graduated cylinders.

c. Snap the neck off a Lead Voluette Ampule Standard Solution, 50 mg/L as Pb.

d. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard to each of three 250-mL samples. Dilute to 250 mL with deionized water. Mix each thoroughly.

e. Analyze each sample as described above. The lead concentration should increase 20 µg/L for each 0.1 mL of standard added.

f. If these increases do not occur, an interference is likely.

Standard Solution Method
Prepare a 10 mg/L lead standard solution by pipetting 10.00 mL of Lead Standard Solution, 100 mg/L, into a 100-mL volumetric flask. Add 0.2 mL of concentrated nitric acid with a TenSette Pipet to prevent adsorption of lead on the flask walls.

Dilute to mark with deionized water and mix well. To make a 120-µg/L Pb standard, pipet 3.00 mL of the 10.0-mg/L standard into 247 mL of deionized water in a 500-mL separatory funnel. Perform the lead procedure as described above.

Interferences

The following do not interfere:

Aluminum	Calcium	Magnesium
Antimony	Chromium	Manganese
Arsenic	Cobalt	Nickel
Cadmium	Iron	Zinc

The following interfere:

Bismuth	Mercury	Tin
Copper	Silver	

Eliminate interference from these metals by the following treatment, beginning after procedure *step 5*.

a. Measure about 5 mL of the prepared dithizone solution into the separatory funnel. Stopper the funnel, invert and open the stopcock to vent. Close the stopcock and shake the solution vigorously for 15 seconds.

b. Allow the funnel to stand undisturbed until the layers separate (about 30 seconds). A yellow, red, or bronze color in the bottom (chloroform) layer confirms the presence of interfering metals.

c. Draw off and discard the bottom (chloroform) layer.

d. Repeat extraction with fresh 5 mL portions of prepared dithizone solution (discarding the bottom layer each time) until the bottom layer shows a pure dark green color for three successive extracts. Extractions can be repeated a number of times without appreciably affecting the amount of lead in the sample.

e. Extract the solution with several 2 or 3 mL portions of pure chloroform to remove any remaining dithizone, again discarding the bottom layer each time.

f. Continue the procedure, substituting 28.5 mL of prepared dithizone solution for the 30 mL in *step 6*.

Large amounts of zinc cause an indistinct color transition at the end point.

Waste Disposal

Both chloroform (D002) and cyanide (D003) solutions are regulated as hazardous wastes by the Federal RCRA. Do not pour these solutions down the drain. Collect chloroform solutions and the cotton plug used in the delivery tubes of the separatory funnel for disposal with laboratory solvent waste. Be sure to store cyanide solutions in a caustic solution with a pH >11 to prevent potential release of hydrogen cyanide gas.

Summary of Method

The DithiVer Metals Reagent is a stable powder form of dithizone. Lead ions in basic solution react with dithizone to form a pink to red lead-dithizonate complex, which is extracted with chloroform.

REQUIRED REAGENTS

Description	Cat. No.
Lead Reagent Set (100 Tests)	22431-00
Includes: (1) 14202-99, (2) 14458-17, (1) 12616-99, (1) 767-14, (1) 2450-53, (2) 2450-26	

Description	Quantity Required Per Test	Units	Cat. No.
Buffer Powder Pillows, Citrate for heavy metals	1 pillow	100/pkg	14202-99
Chloroform, ACS	50 mL	4 L	14458-17
DithiVer® Metals Reagent Powder Pillows	1 pillow	100/pkg	12616-99
Potassium Cyanide, ACS	2 g	113 g	767-14
Sodium Hydroxide Solution, 5.0 N	5 mL	1000 mL	2450-53
Sodium Hydroxide, 5.0 N	drops.	50 mL DB	2450-26
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Clippers, for opening powder pillows	1	each	968-00
Cotton balls, absorbent	1	100/pkg	2572-01
Cylinder, mixing graduated, 50 mL	1	each	1896-41
Cylinder, graduated, 5 mL	1	each	508-37
Cylinder, graduated, 250 mL	1	each	508-46
Funnel, separatory, 500 mL	1	each	520-49
Ring, support, 4-in.	1	each	580-01
Spoon, measuring, 1.0 g	1	each	510-00
Stand, support, 5 x 8 in.	1	each	563-00
Stopper, hollow, No. 1	2	6/pkg	14480-01

OPTIONAL REAGENTS

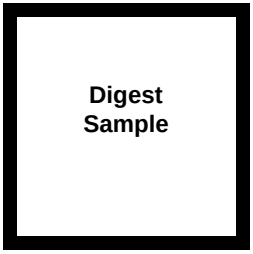
Chloroform, ACS	4 L	14458-17
Lead Standard Solution, 100 mg/L Pb	100 mL	12617-42
Lead Standard Solution, Voluette™ Ampuls, 50 mg/L Pb, 10 mL	16/pkg	14262-10
Nitric Acid Solution, 1:1	500 mL	2540-49
Nitric Acid, ACS	500 mL	152-49
Sodium Hydroxide Standard Solution, 5.0 N	100 mL SCDB	2450-32

OPTIONAL APPARATUS

Description	Units	Cat. No.
Flask, Erlenmeyer, 500 mL	each	505-49
Flask, filtering, 500 mL	each	546-49
Flask, volumetric, Class A, 100 mL	each	14574-42
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable	each	51700-10
Pipet Filler, safety bulb	each	14651-00
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet, serological, 2 mL	each	532-36
Pipet, volumetric, Class A, 2.00 mL	each	14515-36

MANGANESE, TOTAL

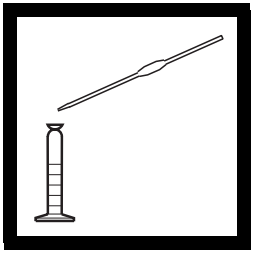
PAN Method*
Range: Liquids: 0.05–3500 mg/L; Solids- 2–35000 mg/kg



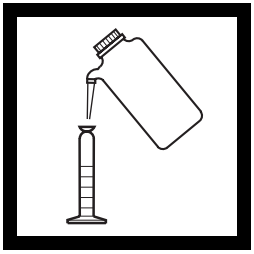
1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *SECTION 3*.

Note: *If samples cannot be analyzed immediately, see Sampling and Storage following these steps.*

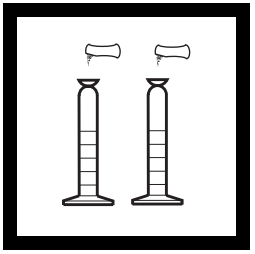
Note: *Rinse all glassware with 1:1 Nitric Acid solution, then rinse with deionized water.*



2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 10-mL mixing cylinder. If the aliquot is more than 0.2 mL, adjust the pH according to the instructions following the digestion method. Dilute to the10-mL mark with deionized water.



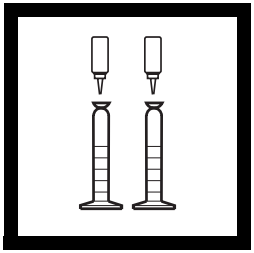
3. Fill a second 10-ml cylinder with deionized water (the blank).



4. Add the contents of one Ascorbic Acid Powder Pillow to each cylinder. Invert to mix.

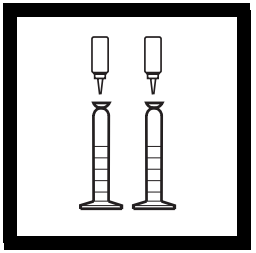
Note: *For samples containing hardness greater than 300 mg/L $CaCO_3$, add four drops of Rochelle Salt Solution to the sample after addition of the Ascorbic Acid Powder Pillow. Hardness can be measured with Hach Water Quality Hardness Test Strips, (Cat. No. 27452-50).*

* Adapted form Goto, K., et al., *Talanta*, 24, 752-3 (1977).



5. Add 15 drops of Alkaline-Cyanide Reagent Solution to each cylinder. Invert to mix.

Note: *A cloudy or turbid solution may form in some samples after addition of the Alkaline-Cyanide Reagent Solution. The turbidity should dissipate after step 6.*

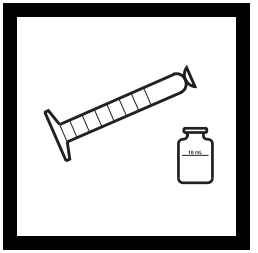


6. Add 21 drops of 0.1% PAN Indicator Solution to each cylinder. Invert to mix.

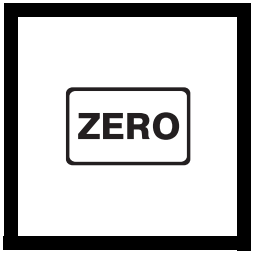
Note: *An orange color will develop if manganese is present.*

7. Begin a two-minute reaction period.

Note: *If the sample contains more than 5 mg/L iron, allow 10 minutes for complete color development. Iron can be measured with Hach Water Quality Iron Test Strips.*



8. After the reaction period, pour the sample and blank into separate 10-mL sample cells.

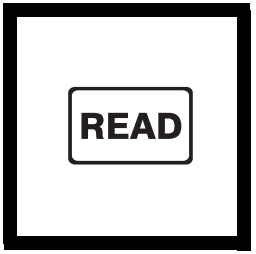


9. Zero the instrument with the blank, using the settings below.

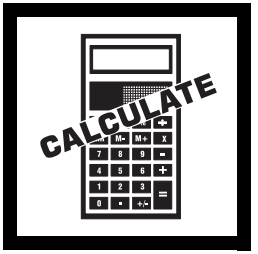
DR/800s
Program No. 43

DR/2010
Program No. 290
560 nm

DR/4000
Program No.2260
560 nm



10. Place the sample in the cell holder. Read the mg/L manganese.



11. Use the equation below the Manganese Sample and Analysis Volume Tables to calculate the true manganese concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Mn Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	DIlute To
0.05-2.1	40.0	8.0	10 mL
0.2-8.7	20.0	4.0	10 mL
0.8-35	10.0	2.00	10 mL
8-350	5.00	0.40	10 mL
80-3500	1.00	0.20	10 mL

Solids

Expected Mn Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
2-175	0.500	8.00	10 mL
5-435	0.400	4.00	10 mL
12-1150	0.300	2.00	10 mL
90-8750	0.200	0.40	10 mL
350-35000	0.100	0.20	10 mL

Calculation For Final Concentration:

$$\frac{A \times 1000}{B \times C} = \text{mg/L or mg/kg Total Mn}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in a clean glass or plastic container. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples can be stored up to six months at room temperature.

Accuracy Check

Standard Additions Method

Note: *Volume accuracy is very important when performing standard additions with 10-mL volumes. The fill mark on the 10-mL sample cell is not intended to measure standard addition volumes.*

a. Perform the manganese method and note the analysis volume used and the mg/L Mn of the sample in *step 10*.

b. Fill three 10-mL graduated mixing cylinders with 10.0 mL of sample.

c. Snap the neck off a Manganese Voluette Ampule Standard, 10 mg/L Mn.

d. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to the three mixing cylinders. Stopper and mix each thoroughly.

e. Analyze each sample as described in the procedure. The manganese concentration should increase 0.1 mg/L for each 0.1 mL of standard added.

f. If these increases do not occur an interference is likely.

An alternative to the above procedure is to measure 10.0 mL of sample into dry sample cells before performing standard additions. A volumetric pipet or a TenSette Pipet can be used to deliver the sample volume.

Standard Solution Method

Prepare a 0.25 mg/L manganese standard solution as follows:

a. Pipet 5.00 mL of Manganese Standard Solution, 1000 mg/L Mn, into a 1000-mL volumetric flask.

b. Dilute to the mark with deionized water. Prepare this solution daily.

c. Pipet 5.00 mL of the above dilution into a 100-mL volumetric flask.

d. Dilute to the mark with deionized water. This second dilution is equivalent to 0.25 mg/L Mn.

Interferences

The following do not interfere up to the indicated concentrations:

Aluminum	20 mg/L
Cadmium	10 mg/L
Calcium	1000 mg/L as $CaCO_3$
Cobalt	20 mg/L
Copper	50 mg/L
Iron	25 mg/L
Lead	0.5 mg/L
Magnesium	300 mg/L as $CaCO_3$
Nickel	40 mg/L
Zinc	15 mg/L

Waste Management

The alkaline cyanide solution contains cyanide. Cyanide solutions should be collected for disposal as reactive (D003) waste. Be sure cyanide solutions are stored in a caustic solution with pH > 11 to prevent release of hydrogen cyanide gas.

Summary of Method

The PAN method is a highly sensitive and rapid procedure for detecting low levels of manganese. An ascorbic acid reagent is used initially to reduce all oxidized forms of manganese to Mn^{2+}. An alkaline-cyanide reagent is added to mask any potential interferences. PAN Indicator is then added to combine with the Mn^{2+} to form an orange-colored complex.

REQUIRED REAGENTS

	Cat. No.
Manganese Reagent Set (50 Tests)	26517-00
Includes: (1) 14577-99, (1) 21223-26, (1) 21224-26, (1) 26058-02, (1) 12263-01	

Description	Quantity Required Per Test	Unit	Cat. No.
Alkaline-Cyanide Reagent	30 drops	50 mL SCDB	21223-26
Ascorbic Acid Powder Pillows	2	100/pkg	14577-99
PAN Indicator Solution, 0.1%	42 drops	50 mL SCDB	21224-26
Water, deionized	10 mL	4 L	272-56

REQUIRED APPARATUS

Cylinder, graduated, mixing, 10 mL	2	each	20886-38

OPTIONAL REAGENTS

Hydrochloric Acid Solution, 1:1 (6 N)	500 mL	884-49
Manganese Standard Solution, 1000 mg/L Mn	100 mL	12791-42
Manganese Standard Solution, PourRite™ Ampule, 10 mg/L Mn, 2 mL	20/pkg	26058-20
Nitric Acid Solution, 1:1	500 mL	2540-49
Nitric Acid, ACS	500 mL	152-49
Rochelle Salt Solution.	29 mL	1725-33
Sodium Hydroxide Solution, 50%	500 mL	2180-49
Water Quality Test Strips, hardness	50/pkg	27452-50
Water Quality Test Strips, iron	25/pkg	27453-25

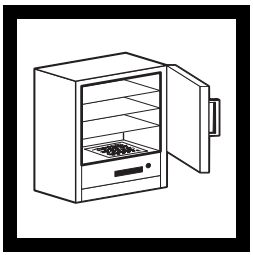
OPTIONAL APPARATUS

Beaker, glass, 1000 mL	each	500-53
Cylinder, mixing, 25 mL	each	20886-40
Dropper, plastic calibrated, 1.0 mL	10/pkg	21247-20
Flask, volumetric, Class A, 1000 mL	each	14574-53
Flask, volumetric, Class A, 100 mL	each	14574-42
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 10.0 mL	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	each	14515-37
Pipet Filler, safety bulb	each	14651-00

MERCURY SAMPLE DIGESTION (33 µg/Kg–10 mg/Kg)

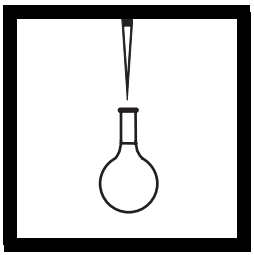
This digestion is to be used for digesting solid samples for use with the Cold Vapor Mercury procedure, Method 10065. For liquid samples, use the digestion presented in the procedure.

Perform this procedure in a fume hood. Toxic gases will be produced

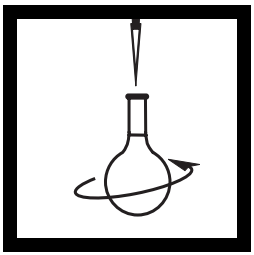


1. Dry the sample at 40 °C or less. Sieve the sample using a 10-mesh (2 mm) sieve.

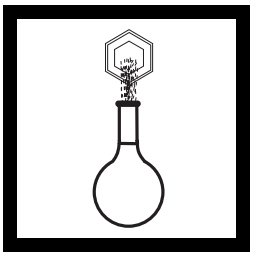
Note: *See Table 1 to select sample size. Drying at greater than 40 °C will cause mercury loss.*



2. Add 10 mL of concentrated nitric acid to a 500-mL round bottom flask.

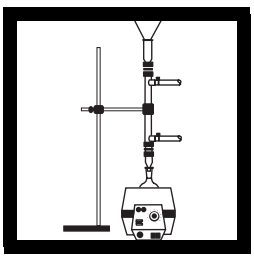


3. Add 10 mL of concentrated sulfuric acid to the flask. Swirl to mix. Cool to room temperature.

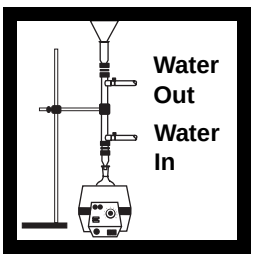


4. See *Table 1* to select the sample size. Add the desired amount of representative sample to the flask. The sample should contain 0.1 to 2.5 µg mercury.

5. Add a magnetic stir bar. Place the flask into a cool electric heating mantle that has stirring capability.



6. Insert a water cooled condenser into the top of the flask. Insert a long stem glass funnel into the top of the condenser to reduce vapor loss.

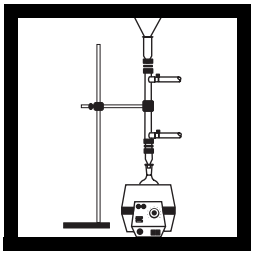


7. Turn on the water to the condenser.

8. Turn on the magnetic stirrer at medium speed and stir the solution without heating for 30 minutes.

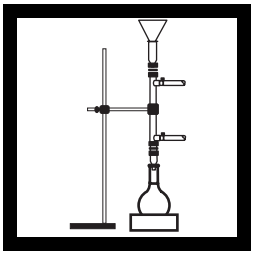
Table 1 Sample Size Selection

Expected Hg Concentration	Suggested Sample Size
1.2 to 10 mg/kg	0.25 g
300 µg/kg to 2 mg/kg	1.00 g
33 µg/kg to 650 µg/kg	3.00g

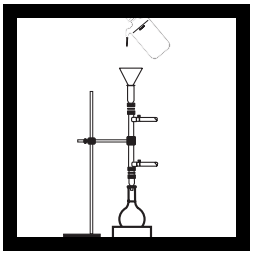


9. Turn the heater on Over a 15-minute period, slowly increase the heat until the solution boils. Avoid rapid heating. Do not heat above the temperature required to boil the solution.

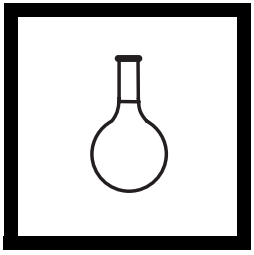
10. Boil the solution for one hour.



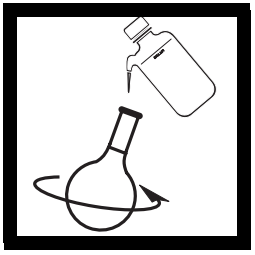
11. After one hour, remove the flask and water cooled condenser from the heater. Cool to room temperature with the flask still attached to the condenser. Keep water flowing through the condenser.



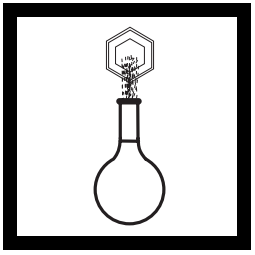
12. When the flask is cool, rinse the funnel and condenser with deionized water into the flask.



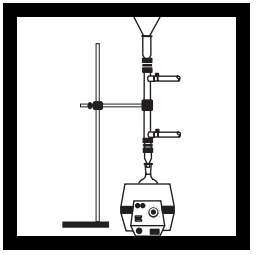
13. Remove the condenser from the flask.



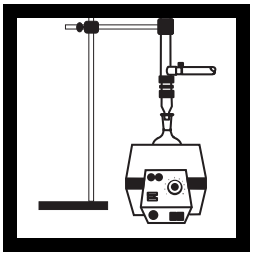
14. Add 50 mL of deionized water to the flask and swirl to mix.



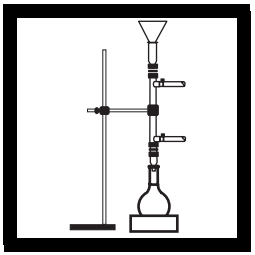
15. Add 0.5 g of potassium permanganate and 0.5 g of potassium persulfate to the cooled solution.



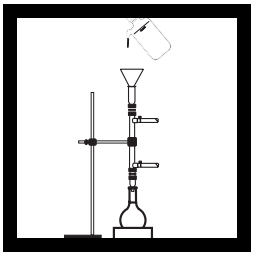
16. Return the flask to the electric heater/stirrer. Insert the condenser with funnel back into the flask. Begin stirring the solution.



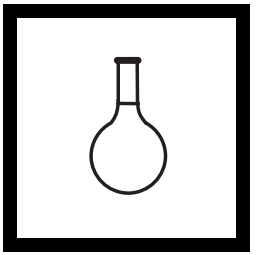
17. Heat the solution to boiling. Boil for 30 minutes. A brown/black precipitate of manganese dioxide will form.



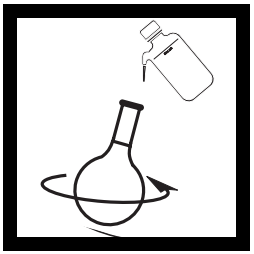
18. Remove the flask and water cooled condenser from the heater. Cool to room temperature with the flask still attached to the condenser. Keep water flowing through the condenser.



19. When the flask is cool, rinse the funnel and condenser with deionized water into the flask.

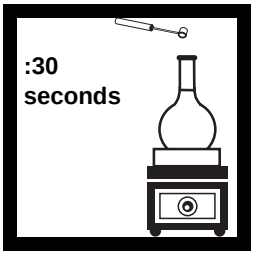


20. Remove the condenser from the flask.



21. Add 50 mL of deionized water to the flask and swirl to mix.

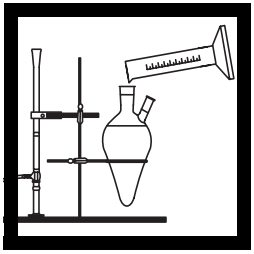
22. Place a cork support ring on a magnetic stirrer. Place the flask on the cork support ring. Begin stirring the solution at medium speed.



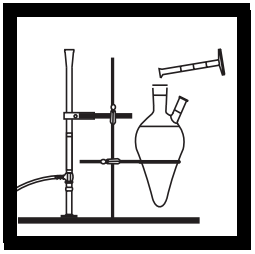
23. Using a 0.5-gram measuring spoon, add hydroxylamine-hydrochloride. Wait at least 30 seconds between each addition. Add hydroxylamine-hydrochloride until the black/brown manganese dioxide precipitate dissolves.

Note: *The sample may contain black/brown particles which are not manganese dioxide.*

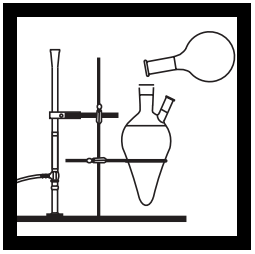
24. Turn the stirrer off. Remove the magnetic stir bar.



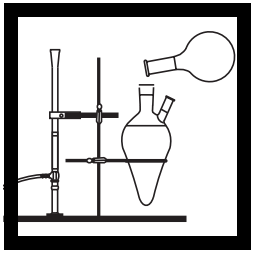
25. Add 800 mL of deionized water to the Cold Vapor Gas Washing Bottle.



26. Add 20 mL of concentrated nitric acid and 40 mL of concentrated sulfuric acid to the Gas Washing Bottle. Swirl to mix.

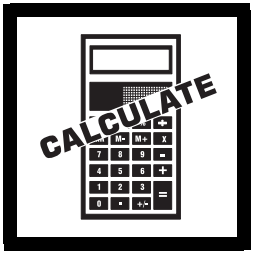


27. Transfer the entire digested sample from the flask to the Gas Washing Bottle.



28. Rinse the flask with 1 N nitric acid and add rinse to the digested sample.

29. Complete the Cold Vapor Mercury Procedure beginning with Phase 2*Phase 2* on page *181*.



30. To calculate the mercury concentration in the solid sample, divide the displayed mercury by the weight of the sample taken for digestion (grams). Multiply the result by 100. Report the value as µg mercury/Kg.

Sampling and Storage

Collect samples in rigorously cleaned glass, fluoropolymer or polyethylene terephthalate (PET) containers (see *Cleaning Containers*, below). Avoid metal containers.

Refrigerate samples immediately after collection and analyze as soon as possible to prevent loss of volatile mercury compounds.

Cleaning Containers

1. Clean containers with detergent and rinse with deionized water.
2. Fill the container almost full of 4 N hydrochloric acid (allow space for thermal expansion of the acid).
3. Heat the container at 65–75 °C for 48 hours.
4. Cool the container to room temperature.
5. Rinse three times with deionized water. Store the container in a sealed plastic bag until ready for use.

Summary of Method

The sample is digested to convert all forms of mercury in the sample to mercuric (Hg^{2+}) ions. The mercuric ions in the digested sample are converted to mercury vapor in a semi-closed system. The vapor is carried into a chemically activated absorber column by ambient air where the mercury vapor is converted to mercuric chloride.

The mercuric chloride is eluted off the column and a sensitive indicator is added. The instrument is zeroed using the absorbance peak of the unreacted indicator. A complexing agent is added to break the mercury/indicator complex. The increase in unreacted indicator causes an increase in absorbance which is proportional to the amount of mercury in the original sample.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Hydroxylamine Hydrochloride, ACS	varies	113 g	246-14
Nitric Acid, ACS	10 mL	500 mL	152-49
Potassium Permanganate, ACS	0.5 g	454 g	168-01
Potassium Persulfate, ACS	0.5 g	454 g	26175-01
Sulfuric Acid, ACS	10 mL	2.5 L	979-09
Water, deionized	120 mL	4 L	272-56

REQUIRED APPARATUS

Bottle, wash, 500 mL	1	each	620-11
Clamp, two-prong extension	1	each	21145-00
Clamp holder	1	each	326-00
Condenser, water cooled	1	each	1806-00
Cork support ring	1	each	27531-00
Cylinder, graduated, 50 mL	1	each	20643-41
Finger cots, Zetex®	2	2/pkg	14647-02
Flask, round-bottom, 500 mL, 24/40 ground glass joint	1	each	27530-00
Funnel, long stem	1	each	549-68
Pipet, serological, 10 mL	1	each	532-38
Pipet Filler, safety bulb	1	each	14651-00
Safety Goggles	1	each	18421-00
Stir Bar, 3/4 x 3/8 in., egg-shaped	1	each	27532-00
Stirrer, magnetic, 120 V	1	each	23444-00
Stirrer, magnetic, 240 V	1	each	23444-02
Support Stand	1	each	563-00
Tubing, latex rubber, ¼-in ID	varies	12 ft.	559-18

Select one based on available voltage:

Heating Mantle, Distillation Apparatus, 115 V	1	each	22744-00
Heating Mantle, Distillation Apparatus, 230 V	1	each	22744-02

OPTIONAL APPARATUS

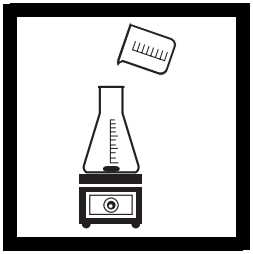
Analytical balance, PocketPro 2060		each	26948-00
Soil sieve, 10-mesh		each	46159-00
Spoon, measuring, 0.5 g		each	907-00
Weighing boat		500/pkg	21790-00

MERCURY

Cold Vapor Mercury Concentration Method*
(0.1 to 2.5 µg/L)

Phase 1 Wastewater Digestion

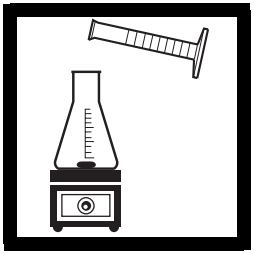
(Must be done in a fume Hood! Toxic gases may be present)



1. Transfer one liter of the sample to a 2000-mL Erlenmeyer flask. Add a 50-mm magnetic stir bar to the sample. Place the flask on a magnetic stirring hot plate and begin stirring.

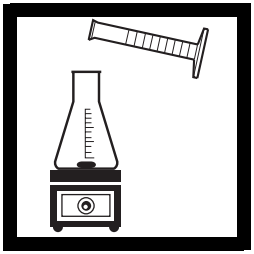
Note: *Solid samples must be digested using the procedure preceding this method. See Solid Sample Digestion For Cold Vapor Mercury on page 173.*

Note: *Hach recommends using dedicated digestion glassware and sample cells for this procedure.*

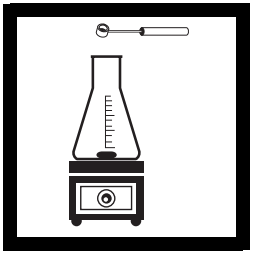


2. Add 50 mL of concentrated sulfuric acid to the sample.

Note: *Determine a reagent blank for each new lot of reagent by running the entire procedure, including the digestion, using one liter of deionized water instead of sample. Add the same amount of potassium permanganate as required by the sample. Subtract the reagent blank from each test result.*



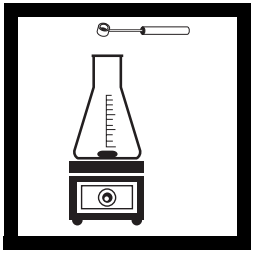
3. Add 25 mL of concentrated nitric acid to the sample.



4. Add 4.0 g of potassium persulfate to the sample. Stir until dissolved

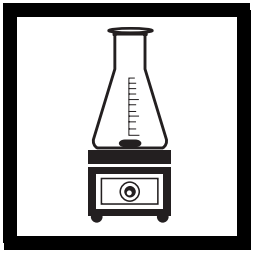
Note: *Alternatively, add one 5-gram measuring scoop of potassium persulfate to the sample.*

* U.S. Patent 5,733,786.



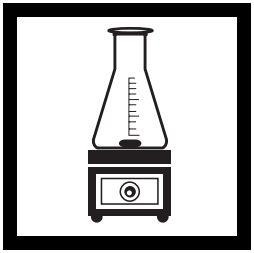
5. Add 7.5 g of potassium permanganate to the sample. Stir until dissolved.

Note: *Alternatively, add a 10-gram measuring scoop of potassium permanganate to the sample.*



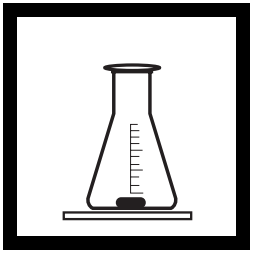
6. Cover the flask with a watch glass. Begin heating the sample to a temperature of 90 °C after the reagents have dissolved. **AVOID BOILING**.

Note: *For a mercury standard or reagent blank in distilled water the heat step is not necessary.*

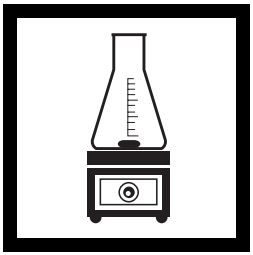


7. Continue to stir and heat the sample at 90 °C for two hours.

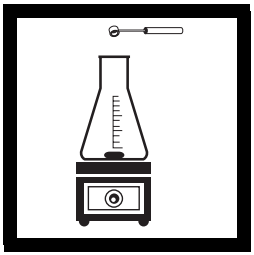
Note: *A dark purple color must persist throughout the two hour digestion. Some samples (sea waters, industrial effluents or samples high in organic matter or chloride concentration) require additional permanganate. It may be difficult to see a dark purple color if the sample contains a black/brown manganese dioxide precipitate. You may add more potassium permanganate if the solution is not dark purple.*



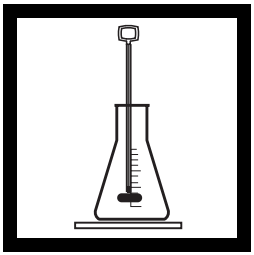
8. Cool the digested sample to room temperature. A brown/black precipitate of manganese dioxide may settle during cooling. If the digested sample does not have a purple color, the digestion may be incomplete. Add more potassium permanganate. Return the sample to the magnetic stirring hot plate and continue digestion until a purple color persists.



9. Return the cool, digested sample to the cool, magnetic stirring hot plate. Turn the stirrer on.



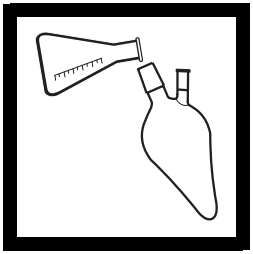
10. Using a 0.5-gram measuring spoon, add 0.5 g-additions of hydroxylamine-hydrochloride until the purple color disappears. Wait 30 seconds after each addition to see if the purple disappears. Add hydroxylamine-hydrochloride until all the manganese dioxide is dissolved.



11. Remove the stir bar.

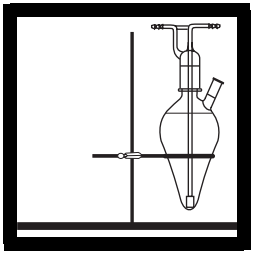
12. The digested sample is now ready for processing by cold vapor separation and preconcentration. Proceed to Phase 2.

Phase 2 Cold Vapor Separation and Pre-concentration of Mercury

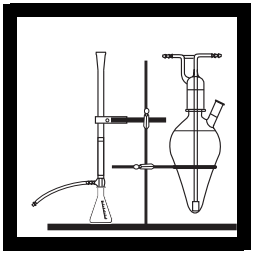


1. Transfer the digested sample to the Cold Vapor Gas Washing Bottle.

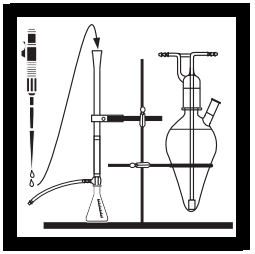
Note: *The volume of digested sample should contain 0.1 to 2.5 µg Hg.*



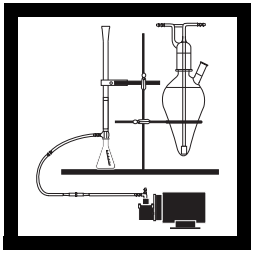
2. Set the Gas Washing Bottle in the support ring. Place the top on the Gas Washing Bottle. Wait until *step 8* to connect the mercury absorber column to the Gas Washing Bottle.



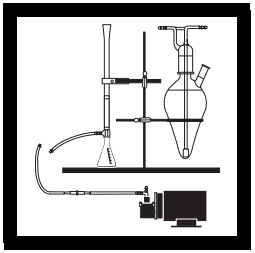
3. Connect the 100-mL Erlenmeyer flask to the Mercury Absorber column.



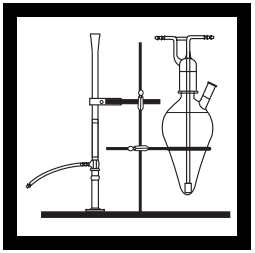
4. Pipet 8 mL of HgEX Reagent B into the Mercury Absorber column.



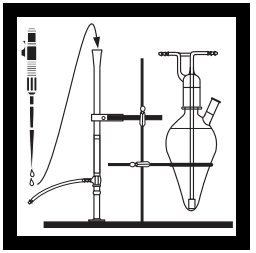
5. Connect the power to the vacuum pump and apply vacuum to the Mercury Absorber Column. Draw most of the HgEX Reagent B into the 100-mL Erlenmeyer flask.



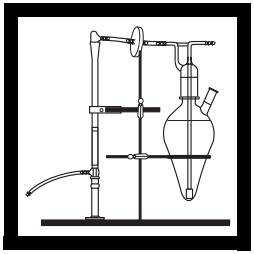
6. Disconnect the vacuum using the quick disconnect when HgEX Reagent B begins to drip from the inner delivery tube on the Mercury Absorber Column (about 10 seconds after starting the vacuum). Do not draw enough air through the column to begin drying the packing.



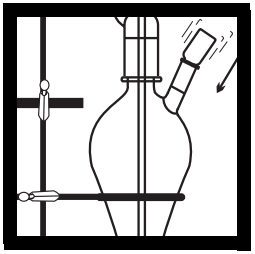
7. Remove the 100-mL Erlenmeyer flask from the Mercury Absorber Column. Replace it with the 10-mL Distilling Receiver.



8. Pipet 2 mL of HgEX Reagent C into the Mercury Absorber Column.

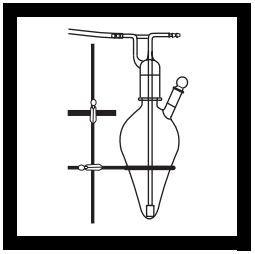


9. Connect the Mercury Absorber Column to the Gas Washing Bottle using the glass elbow.

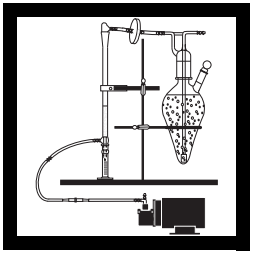


10. Shake an ampule of HgEX Reagent A to suspend the undissolved reagent. Open the ampule and gently shake the contents into the Gas Washing Bottle through the side neck.

Note: *Shaking the ampule is not necessary if there is no undissolved reagent in the ampule.*



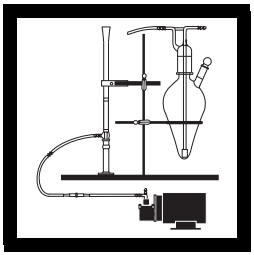
11. Stopper the side neck on the Gas Washing Bottle.



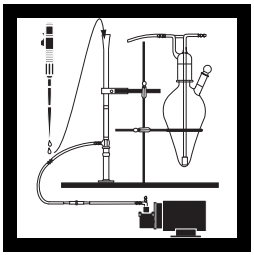
12. Reconnect the vacuum to the Mercury Absorber Column using the quick disconnect. The vacuum will pull HgEX Reagent C through the Mercury Absorber Column packing into the 10-mL receiver. Air bubbles should be produced at the gas dispersion tube in the Gas Washing Bottle.

13. Begin a five-minute reaction period. Let the solution bubble for this period.

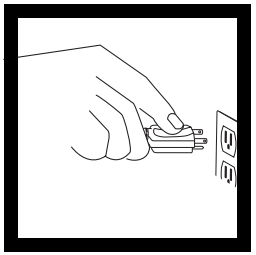
Note: *Air flow rate through the Gas Washing Bottle should be 1-5 liters per minute. Allow more time for lower air flow rates (i.e., if air flow rate is 1 liter/minute, let the solution bubble for 10 minutes).*



14. After the timer beeps, remove the glass elbow from the top of the Mercury Absorber Column. Keep the vacuum pump on.

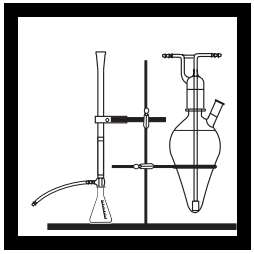


15. Pipet 8 mL of HgEx Reagent B into the Mercury Absorber Column to elute the captured mercury. Continue to apply vacuum to pull the HgEx Reagent B into the Distilling Receiver. Use this distillate in the next phase.

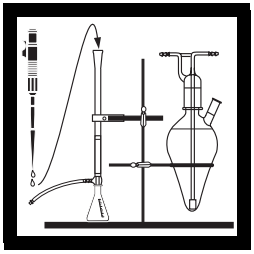


16. Turn off or disconnect power to the vacuum pump when the volume in the Distilling Receiver reaches the 10-mL mark.

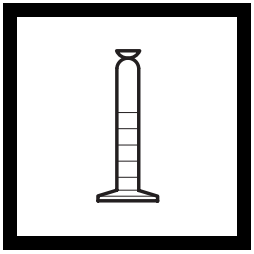
Note: *If necessary, bring the volume in the Distilling Receiver up to 10 mL with HgEX Reagent B. To avoid low volumes, disconnect the vacuum a little sooner in step 6. This leaves more HgEX Reagent B in the packing of the Mercury Absorber Column.*



17. Remove the Distilling Receiver from the Mercury Absorber Column. Reconnect the 100-mL Erlenmeyer flask to the column.



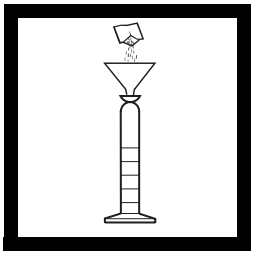
18. Pipet 3 mL of HgEX Reagent B into the Mercury Absorber Column (do not apply vacuum). This keeps the absorber packing wet between tests.



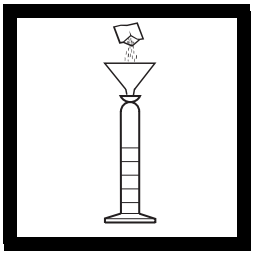
19. The Mercury Absorber Column eluate in the Distilling Receiver is ready for analysis.

Proceed to Phase 3.

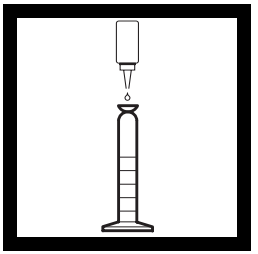
Phase 3 Colorimetric Analysis



1. Using the funnel provided, add the contents of one HgEx Reagent 3 foil pillow to the eluate in the Distilling Receiver. Stopper the receiver. Invert the receiver to dissolve the reagent.

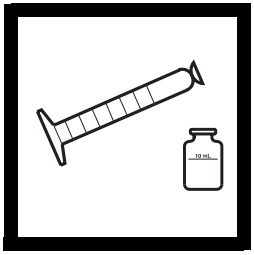


2. Add the contents of one HgEX Reagent 4 foil pillow to the Distilling Receiver using the funnel provided. Stopper the receiver. Invert the receiver to dissolve the reagent.



3. Add 8 drops of HgEX Reagent 5 to the Distilling Receiver. Stopper the Receiver. Invert to mix.

4. Begin a two minute reaction period.



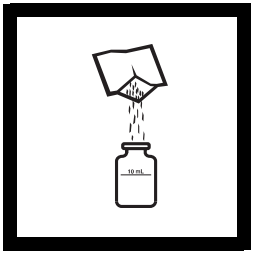
5. During the reaction period, transfer the solution to a 10-mL sample cell. Wipe the sample cell sides with a clean tissue.

6. After the reaction period, zero the instrument with the blank, using the settings below.

DR/800s
Program No. NA

DR/2010
Program No. 312
412 nm

DR/4000
Program No. 2270
412 nm



7. Remove the cell from the cell holder. Add the contents of one HgEx Reagent 6 foil pillow to the solution. Swirl the cell until the reagent is completely dissolved. Immediately go to *step 8*.

Note: *Do not use the funnel to add HgEx Reagent 6 to the sample cell. Any HgEx Reagent 6 in the funnel will make mercury undetectable in subsequent tests.*

8. Return the sample cell to the cell holder. Read the µg/L mercury. This is the concentration of mercury in the original sample.

Sampling and Storage

Collect 1000 mL of sample in an analytically clean, glass or polyethylene terephthalate (PET) container. Add 10 mL of concentrated hydrochloric acid to preserve the sample before sample collection. Fill the container completely full to minimize air space when closed. Close a glass container with a ground glass stopper. Close a PET container with a PET cap or a polypropylene cap (no liner).

Store aqueous samples at 2–6 °C. Acid-preserved samples are stable for at least six months.

Accuracy Check

Standard Additions Method

a. Use a TenSette® pipet to add 0.10 mL of a 12.5 mg/L Mercury Standard Solution to the purged solution in the Gas Washing Bottle after an analysis has been performed. Immediately stopper the Gas Washing Bottle.

b. Begin at *step 3* of *Phase 2*. Follow the procedure steps.

c. Test the eluate as described in Phase 3. The displayed concentration should be between 1.1–1.4 µg/L Hg.

Standard Solution Method

a. Transfer 800 mL of deionized water into the Gas Washing Bottle.

b. Add 50 mL of concentrated sulfuric acid and 25 mL of concentrated nitric acid to the water. Swirl to mix.

c. Prepare a 0.1-mg/L mercury standard solution by serially diluting a 1000-mg/L Mercury Standard Solution:

- To make a 10.0-mg/L standard, add 1.0 mL of concentrated nitric acid to a 500-mL volumetric flask. Dilute 5.00 mL of a 1000 mg/L standard to 500 mL with deionized water. Mix well.

- To make a 1.0-mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100-mL volumetric flask. Dilute 10.00 mL of the 10.0-mg/L standard to 100 mL with deionized water. Mix well.
- To make a 0.1-mg/L standard solution, add 0.2 mL of concentrated nitric acid to a 100-mL volumetric flask. Dilute 10.00 mL of the 1.0-mg/L standard to 100 mL with deionized water. Mix well.

d. Pipet 10.0 mL of the 0.1-mg/L mercury standard solution into the Gas Washing Bottle. Swirl to mix.

e. Begin at *step 2* of *Phase 2* Follow the procedure steps.

f. Test the eluate as described in *Phase 3*. The displayed concentration should be between 0.9-1.1 µg/L Hg.

System Start Up *Phase 2*

Hach recommends that the analyst perform a few analyses on mercury standards and blanks for system equilibration before beginning sample testing. This allows the system to stabilize before processing samples.

Startup Standard

Test a mercury standard solution by following the procedure under *Accuracy Check* using the Standard Solution Method. Continue with *step a* (below) if the value is not within specified limits.

a. Pipet 10.0 mL of the 0.1-mg/L mercury standard solution into the purged solution in the Gas Washing Bottle. Immediately stopper the Gas Washing Bottle.

b. Begin at *step 3* of *Phase 2*. Follow the procedure steps.

c. Test the eluate as described in *Phase 3* The displayed concentration should be between 0.9-1.1 µg/L Hg. Repeat *steps g-i* if the value is not within these limits.

Startup Blank
Run a system blank by using the purged solution in the Gas Washing Bottle after a satisfactory test of the Startup Standard has been completed.

a. Leave the purged solution in the Gas Washing Bottle. Do not add an aliquot of mercury standard.

b. Begin at *step 3* of *Phase 2* Follow the procedure steps.

c. Test the eluate as described in *Phase 3*. The displayed concentration should be ≤0.2 µg/L Hg. Repeat the Startup Blank procedure until a reproducible value is obtained.

Interferences

Standards were used to prepare a single test solution with the following matrix. A second test solution containing only mercury at the same concentration was prepared as the control. The two solutions were digested then analyzed concurrently. There was no interference from the matrix of the test solution at the concentrations listed:

Ion or Substance	Concentration	Ion or Substance	Concentration
Ag^{+}	7 mg/L Ag^{+}	Fe^{+2}	100 mg/L Fe^{2+}
Al^{+3}	10 mg/L Al^{3+}	Hg^{+2}	1 µg/L Hg^{2+}
Au^{+3}	500 µg/L Au^{3+}	Mo^{+6}	10 mg/L Mo^{6+}
Cd^{+2}	10 mg/ L Cd^{2+}	Ni^{+2}	10 mg/L Ni^{2+}
Co^{+2}	10 mg/L Co^{2+}	NO_3^{-}-N	50 mg/L NO_3^{-}-N
Cr^{+6}	10 mg/L Cr^{6+}	Pb^{2+}	10 mg/L Pb^{2+}
Cu^{+2}	10 mg/L Cu^{2+}	SiO_2	100 mg/L SiO_2
F^{-}	1.0 mg/L F^{-}	Zn^{+2}	10 mg/L Zn^{2+}

In addition, no interference occurred with a test solution containing 1000 mg/L Na^{+}, 1000 mg/L K^{+}, 1000 mg/L Mg^{2+}, and 400 mg/L Ca^{2+}.

Storage and Maintenance of the Cold Vapor Mercury Apparatus

Storage

Store the apparatus as follows for fastest system stabilization and greatest sensitivity:

- Store the Gas Washing Bottle filled with deionized water containing 15 mL of concentrated sulfuric acid. Seal the bottle with the Gas Washing Bottle stopper and top.
- Store the Mercury Absorber Column with the packing wetted with HgEX Reagent B. The Erlenmeyer flask should be kept attached underneath the column. The top of the Mercury Absorber column should be attached to the Gas Washing Bottle with the glass elbow as in the procedure.

Glassware Care

Hach recommends using dedicated glassware and sample cells because of the sensitivity of this procedure. Thoroughly clean the glassware and sample cells between tests. After washing, rinse with 1:1 hydrochloric acid solution, then rinse several times with deionized water.

Maintaining the System

- With proper care and storage, the Mercury Absorber Column may be used an unlimited number of times.
- Replace the Mercury Scrubber in the air trap housing at least once for every reagent set used.
- Moisture build up on the Gas Washing Bottle side of the Acro® 50 Vent Filter will reduce the purging air flow rate. If this occurs replace the filter or dry it in an oven at 110 °C.

Summary of Method

The sample is digested to convert all forms of mercury in the sample to mercuric (Hg^{2+}) ions. The mercuric ions in the digested sample are converted to mercury vapor in a semi-closed system. The vapor is carried into a chemically activated absorber column by ambient air where the mercury vapor is converted to mercuric chloride.

The mercuric chloride is eluted off the column and a sensitive indicator is added. The instrument is zeroed using the absorbance peak of the unreacted indicator. A complexing agent is added to break the mercury/indicator complex. The increase in unreacted indicator causes an increase in absorbance which is proportional to the amount of mercury in the original sample.

Waste Disposal

Proper management and disposal of waste is the responsibility of the waste generator. Hach Company provides waste disposal information as a guideline only. It is up to the generator to arrange for proper disposal and comply with applicable local, state, and federal regulations governing waste disposal. Hach Company makes no guarantees or warranties, express or implied, for the waste disposal information represented in this procedure.

1. Dispose of the solution in the Gas Washing Bottle by neutralizing the solution to a pH of 6–9 and flushing to the sanitary sewer with water for several minutes.

2. The mercury contained in one liter of sample is concentrated by a factor of 100 by the Mercury Absorber Column. Mercury analysis within the range of the test may produce a solution in the sample cell that is above the RCRA Toxicity Characteristic limit of 0.20 mg/L Hg. The sample cell will contain 0.25 mg/L mercury if the original sample was at 2.5 µg/L mercury (the upper limit of the test range). Dispose of the solution in the sample cell as a hazardous waste if the test result was greater than 2 µg/L mercury in the original sample. Otherwise, pour the solution into the sanitary sewer and flush with water for several minutes.

3. The mercury scrubber will capture mercury vapor if the Mercury Absorber Column is not properly activated with the HgEX Reagent B and HgEx Reagent C. In addition, mercury is also captured if the capacity of the Absorber Column is exceeded. If the Mercury Scrubber has captured mercury vapor, it must be disposed of according to applicable regulations.

REQUIRED REAGENTS

Description	Cat. No.
Cold Vapor Mercury Reagent Set (25 tests)	26583-00

Description	Quantity Required Per Test	Unit	Cat. No.
HgEx™ Reagent A, Stannous Sulfate Solution Ampules	1	25/pkg	26588-25
HgEx™ Reagent B, Sulfuric Acid Solution	19 mL	500 mL	26589-49
HgEx™ Reagent C, Sodium Hypochlorite Solution	2 mL	55 mL	26590-59
HgEx™ Reagent 3, Alkaline Reagent Powder Pillows	1	25/pkg	26584-48
HgEx™ Reagent 4, Indicator Powder Pillows	1	25/pkg	26585-48
HgEx™ Reagent 5, Hydroxide Solution	8 drops	10 mL SCDB	26586-36
HgEx™ Reagent 6, Complexing Reagent Pillows	1	25/pkg	26587-48
Mercury Scrubber	2/reagent set	2/pkg	26558-00
Digestion Reagents			
Hydroxylamine Hydrochloride, ACS	varies	113 g	246-14
Nitric Acid, ACS	25 mL	500 mL	152-49
Potassium Permanganate, ACS	varies	454 g	168-01
Potassium Persulfate, ACS	4.0 g	454 g	26175-01
Sulfuric Acid, ACS	50 mL	2.5 L	979-09

REQUIRED APPARATUS

Cold Vapor Mercury Apparatus Set	1	each	26744-00
Acro® 50 Vent Filter	1	18/pkg	26833-18
Air Trap Housing	1	each	26639-00
Ampule Breaker	1	each	25640-00
Breaker/Capper Tool for Mercury Scrubber	1	each	26640-00
C-flex Tubing, 0.25" ID, white	4 ft	25 ft	23273-67
Clamp for Mercury Absorber Column	1	each	26562-00
Clamp, Rod	2	each	326-00
Cylinder, 50 mL	1	each	508-41
Distilling Receiver, 10 mL	1	each	26554-38
Erlenmeyer Flask, 100 mL	1	each	26553-42
Funnel	1	each	25843-35
Gas Washing Bottle, 1200 mL	1	each	26622-00
Glass Elbow, with hose adapter	1	each	26552-00
Mercury Absorber Column	1	each	26555-10
Pump, vacuum, 115 V	1	each	14697-00
Pump, vacuum, 220 V	1	each	14697-02
Support Ring for Gas Washing Bottle	1	each	26563-00
Stopper, for Distilling Receiver	1	each	26559-00
Stopper, for Gas Washing Bottle	1	each	26623-00
Support Base and Rod	1	each	329-00

REQUIRED APPARATUS (continued)

Description	Quantity Required Per Test	Unit	Cat. No.
TenSette® Pipet, 0.1–1.0 mL	1	each	19700-01
TenSette® Pipet, 1.0–10.0 mL	1	each	19700-10
TenSette® Pipet tips, for 19700-01	varies	50/pkg	21856-96
TenSette® Pipet tips, for 19700-10	varies	50/pkg	21997-96
Tubing Quick Disconnect, HDPE	1	12/pkg	14810-00
Digestion Apparatus			
Flask, Erlenmeyer, 2000 mL	1	each	24894-54
Hot Plate/Stirrer, 120 V	1	each	23442-00
Hot Plate/Stirrer, 240 V	1	each	23442-02
Spoon, measuring, 0.5 g	1	each	907-00
Stir Bar	1	each	20953-55
Thermometer, -20 to 110 °C	1	each	566-01
Watch Glass	1	each	578-67

OPTIONAL REAGENTS

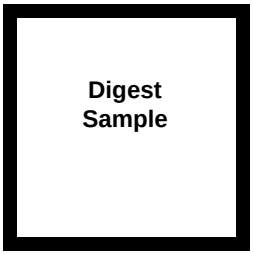
Hydrochloric Acid, ACS	500mL	134-49
Mercury Standard Solution, 12.5 mg/L Hg (NIST)	100 mL	2389-42
Mercury Standard Solution, 1000 mg/L Hg (NIST)	100 mL	14195-42
Water, deionized	4 L	272-56

OPTIONAL APPARATUS

Analytical Balance, 115 V	each	26103-00
Analytical Balance, 230 V	each	26103-02
Cylinder, graduated, 1000 mL, with handle	each	26129-53
Flask, volumetric, Class A, 500 mL	each	14574-49
Flask, volumetric, Class A, 100 mL	each	14574-42
Incoming Air Filtration Apparatus	each	26846-00
Pipet, volumetric, Class A, 10.00 mL	each	14515-38
Pipet, volumetric, Class A, 5.00 mL	each	14515-37
Pipet, volumetric, Class A, 1.00 mL	each	14515-35
Pipet Filler, safety bulb	each	14651-00
Spoon, measuring, 5 g	each	26572-05
Spoon, measuring, 10 g	each	26572-10
Stir Bar Retriever	each	15232-00

NICKEL, TOTAL

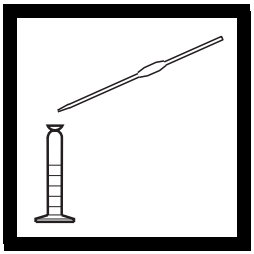
1-(2 Pyridylazo)-2-Naphthol (PAN) Method*
Range: Liquids— 0.05–5000 mg/L; Solids— 3.3–50000 mg/kg



1. Select sample amount from tables following these steps specific to the instrument being used (these tables are different). Digest the sample according to the procedure in *Section 3*.

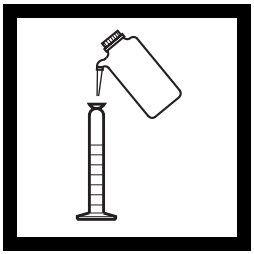
Note: *If sample cannot be analyzed immediately, see* Sample Collection and Preservation following *theses steps.*

Note: *The DR/2010 method uses a 10-mL sample size. The DR/4000 and DR/800 use a 25-mL sample size.*



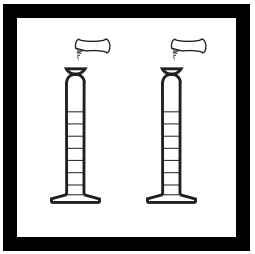
2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 10-mL or 25-mL mixing cylinder, whichever is appropriate. If the aliquot is more than 0.2 mL (0.5 mL for 25-mL sample size), adjust the pH according to the instructions following the digestion method. Dilute to the 10-ml (or 25-mL) mark with deionized water.

Note: *For proof of accuracy, use a 0.5 mg/L Nickel Standard Solution (see Accuracy Check) in place of the sample.*



3. Fill a second 10-mL or 25-mL graduated mixing cylinder with deionized water (the blank).

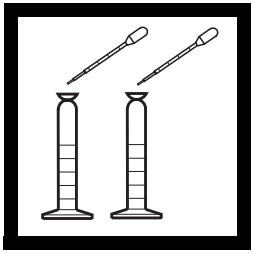
Note: *If sample is less than 10 °C (50 °F), warm to room temperature before analysis.*



4. Add the contents of one Phthalate-Phosphate Reagent Powder Pillow for the appropriate sample size used to each cylinder. Stopper. Invert vigorously until all the powder dissolves.

Note: *If sample contains iron (Fe^{3+}), all the powder must be dissolved completely before continuing with step 5.*

* Adapted form Watanabe, H., *Talanta*, 21 295 (1974).

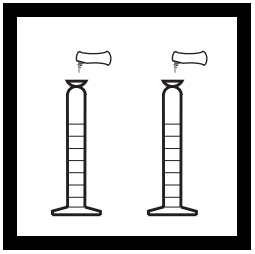


5. Add 0.5 mL of 0.3% PAN Indicator Solution (1 mL for 25-mL samples) to each cylinder. Stopper. Invert several times to mix.

Note: *Use the plastic dropper provided.*

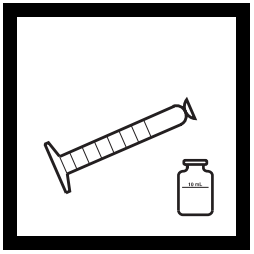
6. Begin a 15-minute reaction period.

Note: *During color development, the sample solution color may vary from yellowish-orange to dark red. The blank should be yellow.*

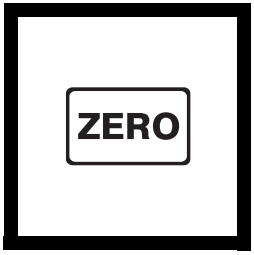


7. When the timer beeps, add the contents of one EDTA Reagent Powder Pillow to each cylinder. Stopper. Invert to dissolve the powder.

Note: *If a precipitate forms, let it settle and decant the solution into a clean sample cell.*



8. Pour the contents of each cylinder into separate 10-mL (or 25-mL) sample cells.



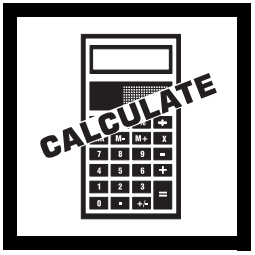
9. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 48
25-mL samples

DR/2010
Program No. 340
560 nm
10-mL samples

DR/4000
Program No.2370
560 nm
25-mL samples

10. Place the sample in the cell holder. Read the mg/L nickel.



11. Use the equation below the Nickel Sample and Analysis Volume Tables to calculate the true nickel concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Note: *There are different equations for different instruments.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

For DR/4000 and DR/800 Instruments

Expected Ni Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.05–3	40.0	20.0	25 mL
0.2–12	20.0	10.0	25 mL
0.65–50	10.0	5.00	25 mL
6.5–500	5.00	1.00	25 mL
65–5000	1.00	0.50	25 mL

$$\frac{A \times 2500}{B \times C} = \text{mg/L Total Ni}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

For DR/2010 Spectrophotometer

Expected Ni Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.05–3	40.0	8.00	10 mL
0.2–12	20.0	4.00	10 mL
0.65–50	10.0	2.00	10 mL
6.5–500	5.00	0.40	10 mL
65–5000	1.00	0.20	10 mL

$$\frac{A \times 1000}{B \times C} = \text{mg/L Total Ni}$$

A = mg/L reading from instrument

B = mL sample amount from table

C = mL analysis volume from table

Solids

For DR/800and DR/4000 Instruments (25 mL sample)

Expected Ni Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
3.3–250	0.500	20.0	25 mL
8.5–625	0.400	10.0	25 mL
22–1650	0.300	5.00	25 mL
165–12500	0.200	1.00	25 mL
650–50000	0.100	0.50	25 mL

$$\frac{A \times 2500}{B \times C} = \text{mg/kg Total Ni}$$

A = mg/L reading from instrument

B = g sample amount from table

C = mL analysis volume from table

For DR/2010 Spectrophotometer (10 mL sample)

Expected Ni Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
3.3–250	0.500	8.00	10 mL
8.5–625	0.400	4.00	10 mL
22–1650	0.300	2.00	10 mL
165–12500	0.200	0.40	10 mL
650–50000	0.100	0.20	10 mL

$$\frac{A \times 1000}{B \times C} = \text{mg/kg Total Ni}$$

A = mg/L reading from instrument

B = g sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Preserved samples can be stored up to six months at room temperature.

Accuracy Check

Standard Additions Method

a. Perform the nickel method and note the analysis volume and mg/L Ni of the sample in *step 10*.

b. Snap the neck off a 300-mg/L Nickel Voluette Ampule Standard. Pipet 5.0 mL of the standard into a 100-mL volumetric flask. Add deionized water to the mark and mix. This is a 15 mg/L Ni solution.

c. Pipet the same analysis volume used for the sample into three 10-mL (or 25-mL) graduated mixing cylinders.

d. Using a Tensette Pipet, add 0.1, 0.2, and 0.3 mL of the 15 mg/L standard into each cylinder, respectively. Dilute to 10 mL (or 25-mL) and mix well.

e. Perform the nickel method beginning with *step 3*. The nickel concentration should increase 0.15, 0.30, and 0.45 mg/L (0.06, 0.12 and 0.18 mg/L for 25 mL samples), respectively, when compared to the value in *step a.*

f. If these increases do not occur, an interference is likely.

Standard Solution Method

Prepare a 0.5 mg/L nickel standard solution by diluting 10.0 mL of a 5 mg/L working stock solution to 100 mL in a 100-mL volumetric flask. Prepare the working stock solution daily by diluting 5.00 mL of Nickel Standard Solution, 1000 mg/L as Ni, to 1000 mL with deionized water.

Or, using the TenSette Pipet, add 0.2 mL of a Voluette Ampule Standard Solution for Nickel, 300 mg/L Ni, into a 100-mL volumetric flask. Dilute to volume with deionized water. This is a 0.6 mg/L standard solution.

Interferences

The following may interfere when present in concentrations exceeding those listed below:

Al^{3+}	32 mg/L
Ca^{2+}	1000 mg/L as ($CaCO_3$)
Cd^{2+}	20 mg/L
Cl^-	8000 mg/L*
Cr^{6+}	40 mg/L
Cu^{2+}	15 mg/L
F^-	20 mg/L
Fe^{3+}	10 mg/L
K^+	500 mg/L
Mg^{2+}	400 mg/L
Mn^{2+}	25 mg/L
Mo^{6+}	60 mg/L
Na^+	5000 mg/L
Pb^{2+}	20 mg/L
Zn^{2+}	30 mg/L

* Monitor chlorine and iron by diluting the sample 1:5, then using Hach Water Quality Test Strips for Chlorine (Cat. No. 27513-40) and Hach Water Quality Test Strips for Iron (Cat. No. 27453-25).

Summary of Method

After buffering the sample and masking any Fe^{3+} with pyrophosphate, the nickel is reacted with 1-(2-Pyridylazo)-2-Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal-PAN complexes except nickel and cobalt. This method is unique because both nickel and cobalt can be determined on the same sample.

REQUIRED REAGENTS

Description	Cat. No.
Nickel Reagent Set, 10 mL sample (100 Tests)	26516-00
Includes: (2) 7005-99, (1) 21502-32, (2) 26151-99	
OR	
Nickel reagent Set, 25-mL sample (100 tests)	22426-00
Includes: (2) 7005-99, (4) 21501-66, (2) 21502-32	

Description	Quantity Required Per Test	Unit	Cat. No.
EDTA Reagent Powder Pillows	2	100/pkg	7005-99
Phthalate-Phosphate Reagent Powder Pillows, 10-mL samples	2	100/pkg	26151-99
P.A.N. Indicator Solution, 0.3%	1 mL	100 mL	21502-32
Water, deionized	10 mL	4 L	272-56

REQUIRED APPARATUS

Cylinder, graduated, mixing, 10 mL	2	each	20886-38

Select one or more based an sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 8.0 mL	1	each	14515-08
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 4.0 mL	1	each	14515-04
Pipet, volumetric, Class A, 2.0 mL	1	each	14515-36
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

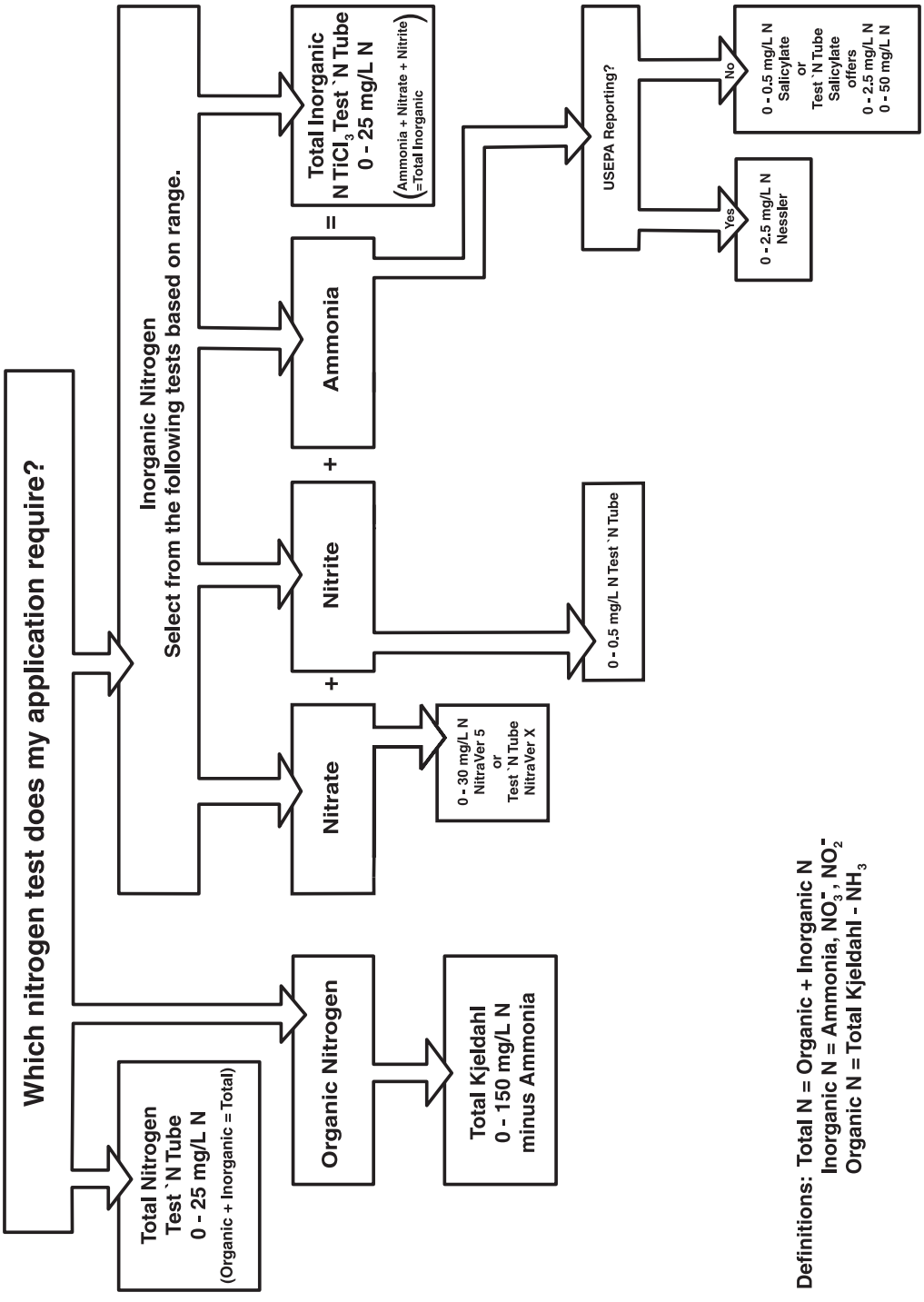
OPTIONAL REAGENTS

Nickel Standard Solution, 1000 mg/L Ni	100 mL	14176-42
Nickel Standard Solution, Voluette™ ampule, 300 mg/L Ni, 10 mL	16/pkg	14266-10
Nitric Acid, ACS	500 mL	152-49
Nitric Acid Solution, 1:1	500 mL	2540-49
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32
Water Quality Test Strips, total chlorine	25/pkg	27513-40
Water Quality Test Strips, total iron	25/pkg	27453-25

OPTIONAL APPARATUS

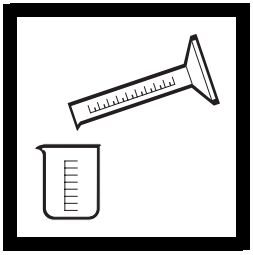
Description	Unit	Cat. No.
Dropper, plastic, calibrated	20/pkg	21247-20
Flask, volumetric, Class A, 100 mL	each	14574-42
Flask, volumetric, Class A, 1000 mL	each	14574-53
Pipet, serological, 1 mL	each	532-35
Pipet, serological, 5 mL	each	532-37
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 5.0 mL	each	14515-37
Pipet, volumetric, Class A,10.0 mL	each	14515-38
Pipet Filler, safety bulb	each	14651-00
Thermometer, -10 to 110 °C	each	1877-01

Selecting the Correct Nitrogen Procedure



NITROGEN, AMMONIA

Nessler Method (with distillation)
Range: 0.06–625 mg/L

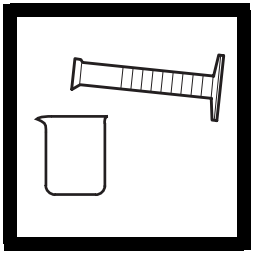


1. Measure 250 mL of water sample using a clean 250-mL graduated cylinder. Pour into a 400-mL beaker.

Note: *Destroy residual chlorine by adding two drops of Sodium Arsenite Solution or one mL of 0.1 N sodium thiosulfate solution for every mg/L of Cl_2. Test the chlorine level with Hach Water Quality Test Strips (Cat. No. 27450-50).*

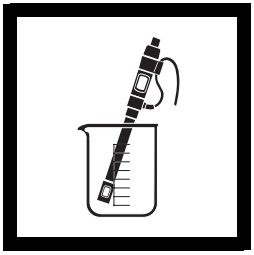
Note: *To make sodium thiosulfate, dissolve 3.5 g of sodium thiosulfate, pentahydrate, in 1 liter of deionized water. Prepare fresh solution weekly.*

Note: *For most accurate results, run a deionized water blank through the distillation and colorimetric procedure. Subtract the blank value from the final reading to obtain the final concentration.*

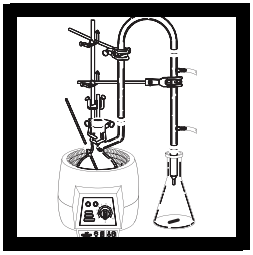


2. Measure 25 mL of Borate Buffer Solution using a 25-mL graduated cylinder. Add it to the beaker and mix.

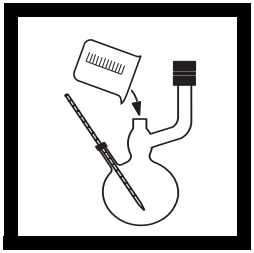
Note: *For proof of accuracy, use a 1.0 mg/L ammonia nitrogen standard (see OPTIONAL REAGENTS) in place of the sample.*



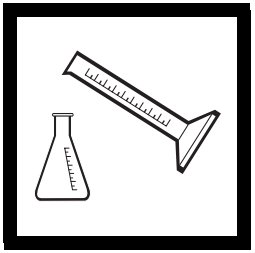
3. Measure the pH of the solution with a pH meter. Using a dropper, add 1 N Sodium Hydroxide Standard Solution drop-wise until the pH is about 9.5.



4. Set up the distillation apparatus by assembling the general purpose accessories as shown in *Distillation Apparatus Manual*. Place a stir bar in the distillation flask.

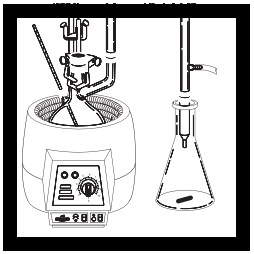


5. Pour the solution into the distillation flask. Cap the distillation flask.

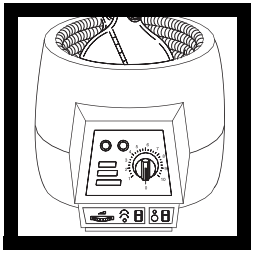


6. Using a 25-mL graduated cylinder, pour 25 mL of deionized water into a clean, 250- mL Erlenmeyer flask.

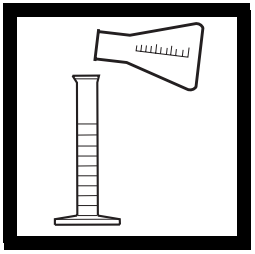
7. Add the contents of one Boric Acid Powder Pillow to the flask. Mix thoroughly by swirling.



8. Place the flask under the drip tube. Elevate the flask so the end of the drip tube extends below the level of the solution in the flask. This may require a laboratory jack.

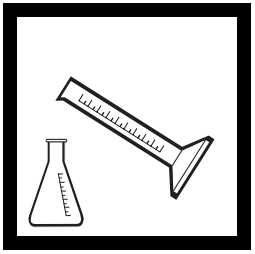


9. Turn on the stirrer power switch. Set the stir control to 5. Set the heater control to 10. Turn on the water and adjust it so a constant flow is maintained through the condenser.

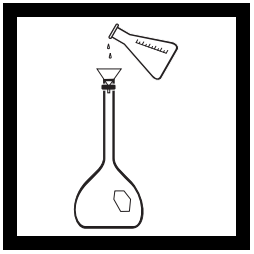


10. Collect 150 mL of distillate, then turn the heater off. **Immediately** remove the Erlenmeyer flask. Using a graduated cylinder, measure the distillate to be sure that 150 mL has been collected (total volume should be 175 mL).

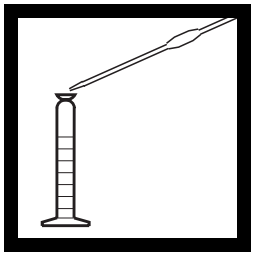
Note: *Remove the flask immediately so the distillate is not drawn back into the distillation flask by vacuum. Or, remove the small glass stopper in the thermometer well to break the vacuum.*



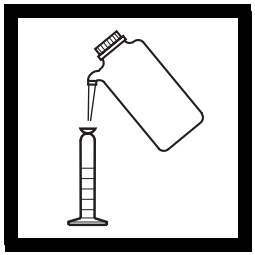
11. Return the distillate to the Erlenmeyer flask. Adjust the pH of the distillate to about 7 by adding 1N Sodium Hydroxide Standard Solution dropwise. Measure the pH with a pH meter.



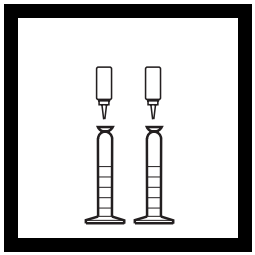
12. Pour the distillate into a 250-mL volumetric flask. Rinse the Erlenmeyer flask with deionized water and add the rinsings to the volumetric flask. Dilute to the mark. Stopper and mix thoroughly by inversion. The distillate is ready for analysis.



13. Select the appropriate analysis volume of the distilled sample given in *Table 1*. Pipet the analysis volume from the sample and the distilled blank into separate 25-mL graduated mixing cylinders.

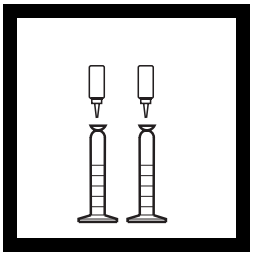


14. Fill another 25-mL graduated cylinder with 25 mL of deionized water.



15. Add three drops of Mineral Stabilizer to each cylinder. Invert several times to mix.

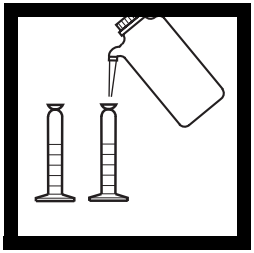
Note: *Hold the dropping bottles upright while dispensing.*



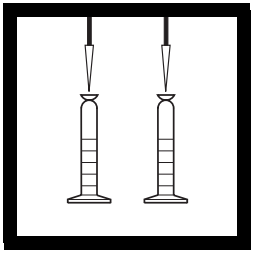
16. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder. Invert several times to mix.

Table 1

Expected NH_3-N Concentration (mg/L)	Sample Analysis Volume (mL)	Dilute To
0.06 – 2.5	25	25 mL
0.3 – 12.5	5	25 mL
1.5 – 62.5	1	25 mL
3 – 125	0.5	25 mL
15 – 625	0.1	25 mL



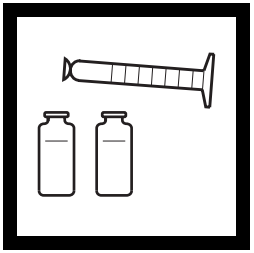
17. Fill both cylinders to the 25-mL mark with deionized water.



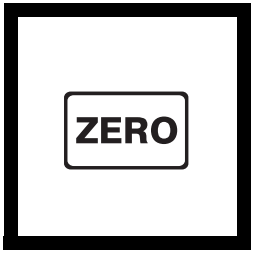
18. Pipet 1 mL of Nesslers Reagent to each cylinder. Stopper, invert repeatedly. The solution should not be hazy

Note: *Any haze (or turbidity) will cause incorrect results.*

19. Begin a one-minute reaction period.



20. After one minute, pour the contents of each cylinder into a 25-mL sample cell.

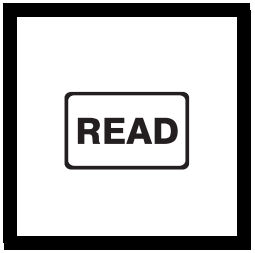


21. Zero the instrument with the reagent blank, using the settings below.

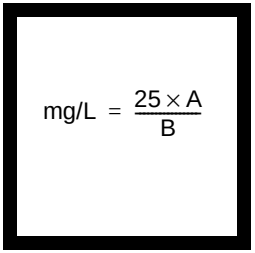
DR/800
Program No. NA

DR/2010
Program No. 380 or user-entered number
425 nm

DR/4000
Program No. 2400
425 nm



22. Place the sample in the cell holder. Read the mg/L ammonia as nitrogen.



23. Use the formula shown to calculate the final mg/L nitrogen.

Where:
A = mg/L read from the display

B = volume of distillate used for analysis

Sampling and Storage

Collect samples in a cleaned glass or plastic container.

If chlorine is present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl_2 in a 1-liter sample. Preserve the sample by reducing the pH to 2 or less with sulfuric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Before analysis, warm samples to room temperature and neutralize with 5 N sodium hydroxide. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method

a. Snap the neck off an Ammonium Nitrogen Voluette Ampule Standard Solution, 50 mg/L NH_3–N.

b. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard to three 25-mL samples. Mix each thoroughly.

c. Analyze each sample as described above beginning at *step 13*. The nitrogen concentration should increase 0.20 mg/L for each 0.1 mL of standard added.

d. If these increases do not occur, an interference is likely.

Standard Solution Method

To check accuracy, use a 1.0 mg/L Ammonium Nitrogen Standard Solution. Or, this can be prepared by diluting 1.00 mL of solution from a 50 mg/L NH_3–N Voluette Ampule Standard for Ammonium Nitrogen to 50.0 mL with deionized water. Prepare this solution daily. Perform the Nessler procedure on 25 mL of this standard beginning at *step 13*.

Interferences

Distillation removes most interferences. However, sulfide will interfere by causing turbidity with Nessler reagent.

Summary of Method

The Mineral Stabilizer complexes hardness in the sample. The Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonium ions. A yellow color is formed proportional to the ammonia concentration.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Borate Buffer Solution	25 mL	1000 mL	14709-53
Boric Acid Powder Pillows	1 pillow	10/pkg	14817-99
Mineral Stabilizer	6 drops	50 mL SCDB	23766-26
Nesslers Reagent	2 mL	500 mL	21194-49
Sodium Hydroxide Solution, 1 N	varies	100 mL	1045-32
Polyvinyl Alcohol Dispersing Agent	6 drops	50 mL SCDB	23765-26
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Beaker, 400 mL	1	each	500-48
Bottle, glass, amber, 1000 mL		6/pkg	7144-63
Cylinder, graduated, 25 mL	1	each	508-40
Cylinder, graduated, 250 mL	1	each	508-46
Cylinder, graduated, mixing, tall-form, 25 mL	2	each	21190-40
Distillation Apparatus, General Purpose Accessories	1	each	22653-00
Distillation Heater and Support Apparatus, 115 V	1	each	22744-00
Distillation Heater and Support Apparatus, 230 V	1	each	22744-02
Dropper, plastic, 0.5 and 1-mL marks	1	20/pkg	21247-20
Flask, volumetric, Class A, 250 mL	1	each	14574-46
Flask, Erlenmeyer, 250 mL	1	each	505-46
pH/ISE Meter, *sens**ion***™*2*, portable	1	each	51725-10
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96

OPTIONAL REAGENTS

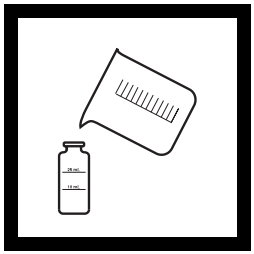
Description	Unit	Cat. No.
Nitrogen Ammonia Standard Solution, 1.0 mg/L as N	500 mL	1891-49
Nitrogen Ammonia Standard Solution, Voluette ampules 50 mg/L as N, 2 mL	20/pkg	14791-20
pH Paper, pH 9.0-12.0	5 rolls/pkg	385-33
Sodium Arsenite Solution, 5 g/L	100 mL MDB	1047-32
Sodium Thiosulfate, pentahydrate, ACS	454 g	460-01
Sodium Thiosulfate Solution, 0.1 N	100 mL MDB	323-32
Sodium Hydroxide Pellets	500 g	187-34
Water Quality Test Strips, Free and Total Chlorine	50/pkg	27450-50

OPTIONAL APPARATUS

Ammonia Combination Electrode Analysis Package	each	23487-00
Balance, AccuLab Pocket Pro	each	25568-00
Bottle, glass dispenser, 118 mL	each	591-00
Bottle, plastic wash, 32-oz. (1000 mL)	each	620-16
Cylinder, graduated, 50 mL	each	508-41
Finger Cots	2/pkg	14647-02
Flask, volumetric, 100 mL, Class A	each	14574-42
Jack, laboratory	each	22743-00
Mini Grinder, 120 Vac	each	20991-00
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Safety Goggles	each	18421-00

NITRATE, HIGH RANGE

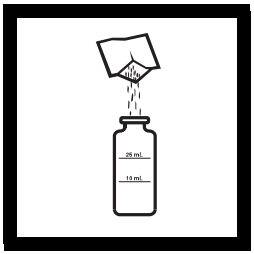
Cadmium Reduction Method
Range: 0 to 30.0 mg/L NO_3^-–N



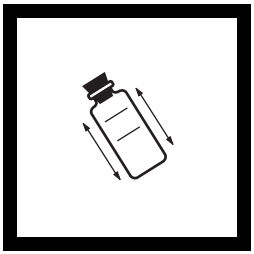
1. Fill a sample cell with 10 or 25 mL of sample.

Note: *Use a 10-mL sample for the DR/4000 and DR/800. Use a 25-mL sample for the DR/2010.*

Note: *A reagent blank must be determined on each new lot of NitraVer 5. Perform steps 1 to 7 using deionized water as the sample. Subtract this value from each result obtained with this lot of reagent.*



2. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow to the cell (the prepared sample). Stopper.

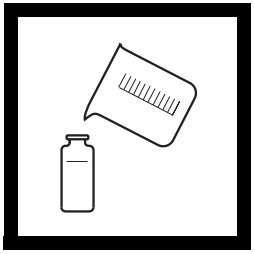


3. Shake the cell vigorously for one minute.

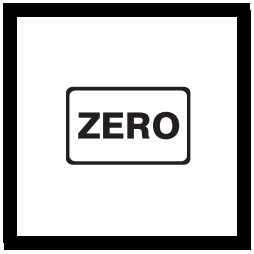
Note: *Shaking time and technique influence color development. For most accurate results, make successive tests on a standard solution. Adjust the shaking time to obtain the correct result.*

4. After shaking, begin a five-minute reaction period.

Note: *An amber color will develop if nitrate nitrogen is present.*



5. During the reaction period, fill another sample cell with 10 or 25 mL of sample (the blank).



6. During the reaction period, zero the instrument with the blank, using the settings below.

DR/800s
Program No. 51

DR/2010
Program No. 355
500 nm

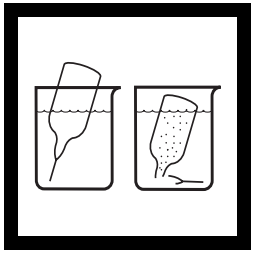
DR/4000
Program No. 2530
500 nm

7. Place the sample in the cell holder. Read the mg/L nitrate nitrogen.

Note: *A cadmium deposit will remain after the NitraVer 5 Nitrate Reagent Powder dissolves and will not affect results.*

Note: *Rinse the sample cell immediately after use to remove all cadmium particles.*

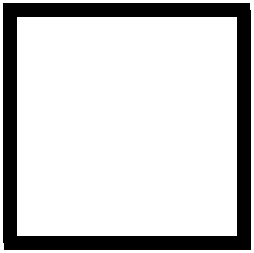
Using AccuVac Ampuls



1. Collect at least 40 mL of sample in a 50-mL beaker. Fill a NitraVer 5 Nitrate AccuVac Ampul with sample.

Note: *Keep the tip immersed while the ampul fills completely.*

Note: *A reagent blank must be determined on each new lot of NitraVer 5. Repeat steps1 to 7 using deionized water as the sample. Subtract this value from each result obtained with this lot of reagent.*



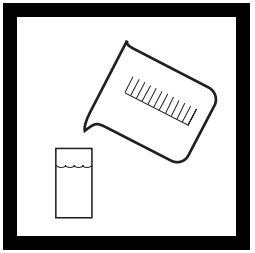
2. Begin a one-minute mixing period. Invert the ampul repeatedly until the timer beeps. Wipe off any liquid or fingerprints.

Note: *Inversion time and technique influence color development. For most accurate results, do successive tests on a 10 mg/L Nitrate Nitrogen Standard Solution. Adjust the inversion time to obtain the correct result. Try starting with 48–52 inversions per minute. Adjust for each new lot of reagent.*

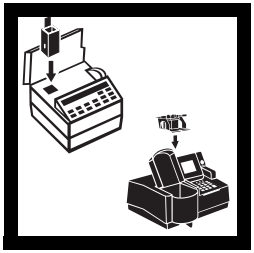
3. Begin a five-minute reaction period.

Note: *A cadmium deposit will remain after the NitraVer 5 Nitrate Reagent Powder dissolves and will not affect results.*

Note: *An amber color will develop if nitrate nitrogen is present.*

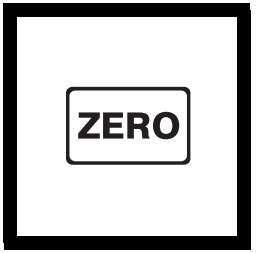


4. During the reaction period, fill a zeroing vial with at least 10 mL of sample (the blank).



5. If necessary, insert an AccuVac Vial Adapter into the cell holder during the reaction period.

Note: *The DR/4000 and DR/2010 Spectrophotometers require AccuVac adapters.*

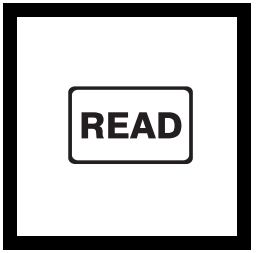


6. During the reaction period. zero the instrument with the blank, using the settings below.

DR/800s
Program No. 50

DR/2010
Program No. 361
500 nm

DR/4000
Program No. 2535
500 nm



7. When the timer beeps, place the sample in the cell holder. Read the mg/L nitrate nitrogen.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For storage periods up to 14 days, adjust sample pH to 2 or less with sulfuric acid, ACS, (about 2 mL per liter). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature. Neutralize the sample with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives. Correct test results for volume additions.

Accuracy Check

Standard Additions Method

a. Snap the neck off a fresh High Range Nitrate Nitrogen Voluette Ampule Standard, 500 mg/L NO_3^--N.

b. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard to three 10- or 25-mL samples. Mix each thoroughly. (For AccuVac ampuls, use 50-mL beakers.)

c. Analyze each sample as described above. For 25-mL samples, the nitrogen concentration should increase 2.0 mg/L for each 0.1 mL of standard added.For 10-mL samples, the nitrogen concentration should increase 5.0 mg/L for each 0.1 mL of standard added.

d. If these increases do not occur, an interference is likely.

Standard Solution Method

Use a 10.0 mg/L Nitrate Nitrogen Standard Solution listed under Optional reagents to check test accuracy. Or, this can be prepared by diluting 1.00 mL of solution from a High Range Nitrate Nitrogen Voluette Ampule Standard Solution, 500 mg/L NO_3^--N, to 50.0 mL with deionized water. Use this standard in place of sample in *step 3* of the procedures.

Interferences

Compensate for nitrite interference as follows:

a. Add Bromine Water, 30 g/L, drop-wise to the sample in *step 3* until a yellow color remains.

b. Add one drop of Phenol Solution, 30 g/L, to destroy the color.

c. Proceed with *step 3*. Report results as total nitrate and nitrite.

Strong oxidizing and reducing substances will interfere. Ferric iron causes high results and must be absent. Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride levels (i.e., seawater), but a

calibration must be performed using standards spiked to the same chloride concentration. Chloride can be measured using Hach Water Quality Test Strips for Chloride (Cat. No. 27449-40).

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.

Summary of Method

Cadmium metal reduces nitrates present in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. This salt couples to gentisic acid to form an amber-colored product.

REQUIRED REAGENTS (Using Powder Pillows)

Description	Quantity Required Per Test	Unit	Cat. No.
NitraVer® 5 Nitrate Reagent Powder Pillows, 25 mL	1	100/pkg	14034-99
NitraVer® 5 Nitrate Reagent Powder Pillows, 10 mL	1	100/pkg	21061-69

REQUIRED REAGENTS (Using AccuVac® Ampuls)

NitraVer 5® Nitrate Reagent AccuVac Ampul	1 ampul	25/pkg	25110-25

REQUIRED APPARATUS (Using Powder Pillows)

Stopper, rubber, size 2	1	12/pkg	2118-02

REQUIRED APPARATUS (Using AccuVac® Ampuls)

Adapter, AccuVac® Vial, DR/2010	1	each	43784-00
Adapter, AccuVac® Vial, DR/4000	1	each	48190-00
Beaker, 50 mL	1	each	500-41
Zeroing Vial	1	each	21228-00

OPTIONAL REAGENTS

Description	Unit	Cat. No.
Bromine Water, 30 g/L	25 mL*	2211-20
Nitrate Nitrogen standard Solution, 10 mg/L NO_3^-–N	500 mL	307-49
Nitrate Nitrogen Standard Solution, Voluette™ Ampule, 500 mg/L (NO_3^-–N), 10 mL	16/pkg	14260-10
Phenol Solution, 30 g/L	29 mL	2112-20
Sodium Hydroxide Standard Solution, 5.0 N	50 mL* SCDB	2450-26
Sulfuric Acid, ACS	500 mL*	979-49
Water, deionized	4 L	272-56
Water Quality Test Strips, Chloride	s40/pkg	27449-40

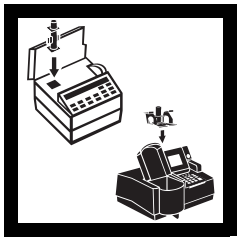
OPTIONAL APPARATUS

AccuVac® Ampul Snapper Kit	each	24052-00
Ampule Breaker Kit	each	21968-00
Cylinder, graduated, 25 mL	each	1081-40
Dropper, for 1-oz. bottle	each	2258-00
Flask, volumetric, Class A, 50 mL	each	14574-41
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable	each	51700-10
Pipet, serological, 2 mL	each	532-36
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00 mL	each	14515-35
Pipet Filler, safety bulb	each	14651-00
Thermometer, -10 to 110 °C	each	1877-01

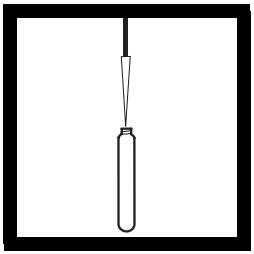
* Contact Hach for larger sizes.

NITRATE, HIGH RANGE

Chromotropic Acid Method
Range: 0–30.0 mg/L NO_3^-–N

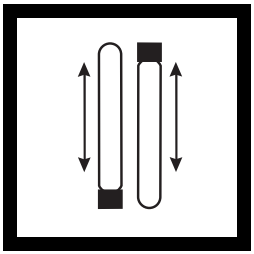


1. Place the COD Vial Adapter into the instrument cell holder.



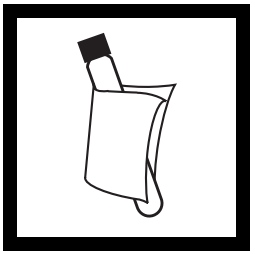
2. Remove the cap from a NitraVer X Reagent A vial and add 1 mL of sample (the sample blank).

Note: *Run a reagent blank for this test. Use Nitrate-free water in place of the sample. Subtract this result from all tests run with this lot of NitraVer X Reagent B. Run a new reagent blank for each new lot.*



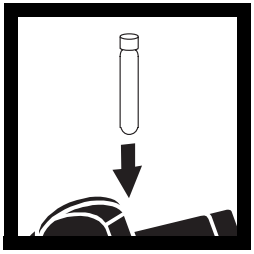
3. Cap the tube and invert 10 times to mix.

Note: *This test is technique sensitive. Low results may occur if these instructions are not followed. Hold the tube vertical with the cap pointing up. Invert the vial so the cap points down. Wait for all of the solution to flow to the cap end. Pause. Return the vial to the original position. Wait for all the solution to flow to the vial bottom. This process equals one inversion. Repeat 10 times.*



4. Clean the outside of the vial with a towel.

Note: *Wiping with a damp towel, followed by a dry one, will remove fingerprints and other marks.*



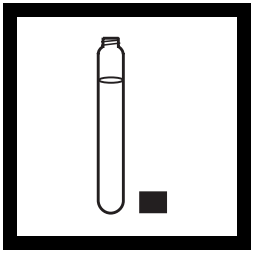
5. Place the sample blank into the vial adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

6. Zero the instrument with the blank, using the settings below.

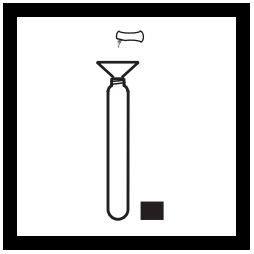
DR/800s
Program No. 57

DR/2010
Program No. 344
410 nm

DR/4000
Program No. 2511
410 nm



7. Remove the vial from the instrument. Remove the cap from the vial.



8. Using a funnel, add the contents of one NitraVer X Reagent B Powder Pillow to the vial. Cap. Invert to mix (this is the prepared sample).

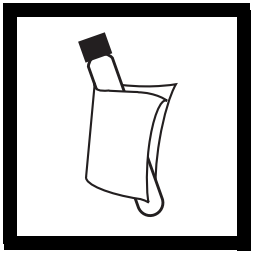
Note: *See step 3 for inversion instructions*

Note: *Some solid matter will not dissolve.*

9. Begin a five-minute reaction period. Do not invert the vial again.

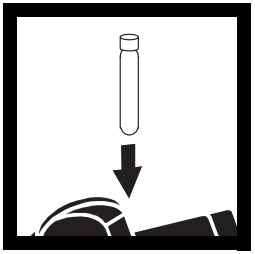
Note: *A yellow color will develop if nitrate nitrogen is present.*

Note: *Complete Steps 10–12 within 5 minutes after the timer beeps.*

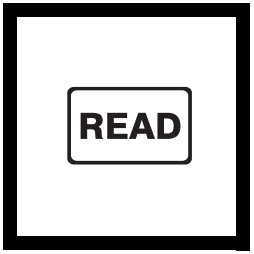


10. When the timer beeps, clean the outside of the vial with a damp towel.

Note: *Wiping with a damp towel, followed by a dry one, will remove fingerprints and other marks.*



11. Place the prepared sample into the vial adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.



12. Place the sample in the cell holder. Read the mg/L nitrate expressed as nitrogen.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods (up to 14 days), adjust sample pH to 2 or less with concentrated sulfuric acid, ACS (about 2 mL per liter). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution. Correct the test result for volume additions.

Do not use mercury compounds as preservatives.

Accuracy Check

Standard Additions Method

a. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.

b. Snap the neck off a fresh High Range Nitrate Nitrogen Voluette Ampule Standard, 500 mg/L NO_3^--N.

c. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard to the three mixing cylinders, respectively. Mix each thoroughly.

d. Analyze each sample as described in the procedure; use a 1-mL aliquot of a spiked sample prepared in Step C in each test. The nitrogen concentration should increase 2.0 mg/L for each 0.1 mL of standard added.

e. If these increases do not occur an interference is likely.

Standard Solution Method

Use a 15.0 mg/L Nitrate Nitrogen Standard Solution (see *Optional Reagents*) to check test accuracy. Or, prepare a standard by pipetting 3.00 mL of solution from a 500 mg/L NO_3^--N High Range Nitrate Nitrogen Standard Solution into a Class A 100-mL volumetric flask. Dilute to the line with deionized water. Substitute this standard for the sample and perform the test as described. The mg/L NO_3^--N reading should be 15 mg/L.

Interferences

Copper is a positive interference at all levels. Copper can be measured with Hach Water Quality Test Strips, Total Copper (Cat. No. 27451-25).

Barium is a negative interference at concentrations greater than 1 mg/L.

Chloride does not interfere below 1000 mg/L. Measure chloride with Hach Quantab Titrators, High Range Chloride (Cat. No. 27513-40).

Hardness does not interfere.

Nitrite is a positive interference at concentrations greater than 12 mg/L. Remove nitrite interference up to 100 mg/L by adding 400 mg of urea (one full 0.5 g Hach measuring spoon) to 10 mL of sample. Swirl to dissolve. Proceed with the Nitrate test. Nitrite can be measured with Hach Water Quality Test Nitrate, Nitrite Test Strips (Cat. No. 27454-25). Some dilution may be necessary.

Summary of Method

Nitrate reacts with chromotropic acid under strongly acidic conditions to yield a yellow product with a maximum absorbance at 410 nm.

REQUIRED REAGENTS

Description	Cat. No.
Test 'N Tube™ NitraVer® X Nitrate Reagent Set (50 vials)	26053-45

Includes: 50 NitraVer® X Reagent A Test 'N Tubes, 50 NitraVer® B Reagent Powder Pillows, and 100 mL deionized water

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
COD Vial Adapter, DR 800	1	each	48464-00
COD Vial Adapter, DR/2010	1	each	44799-00
CDO Vial Adapter, DR/4000	1	each	48189-00
Funnel, micro	1	each	25843-35
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	varies	50/pkg	21856-96
Test Tube Rack	1-3	each	18641-00

OPTIONAL REAGENTS

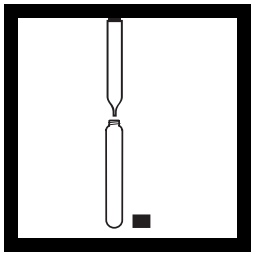
Nitrate-Nitrogen Standard Solution, 15 mg/L NO_3^--N	100 mL MDB	24151-32
Nitrate-Nitrogen Standard Solution, Voluette™ ampule, 500 mg/L N	16/pkg	14260-10
Quantab Titrators for High range Chloride	40/pkg	27513-40
Sodium Hydroxide Standard Solution, 5.0 N	50 mL DB	2450-26
Sulfuric Acid, ACS	500 mL	979-49
Urea, ACS	100 g	11237-26
Water, deionized	4 L	272-56
Water Quality Test Strips, total copper	25/pkg	27451-25
Water Quality Test Strips, nitrate, nitrite	25/pkg	27454-25

OPTIONAL APPARATUS

Ampule Breaker Kit	each	24846-00
Cylinder, graduated, mixing, 25-mL	each	26363-40
Flask, volumetric, Class A, 100 mL	each	14574-42
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipet, serological, 2 mL	each	532-36
Pipet, volumetric, Class A, 3.00 mL	each	14515-03
Spoon, measuring, 0.5 g	each	907-00

NITRITE, TEST 'N TUBE

Diazotization Chromotropic Acid Method
USEPA- approved for reporting wastewater analyses
Range: 0–0.500 mg/L NO_2^-–N

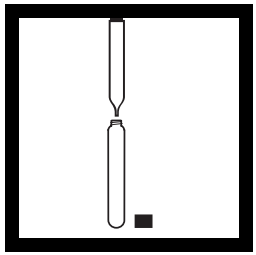


1. Fill a Test 'N Tube NitriVer 3 Nitrite vial with 5 mL of sample. Cap and shake to dissolve powder. This is the prepared sample.

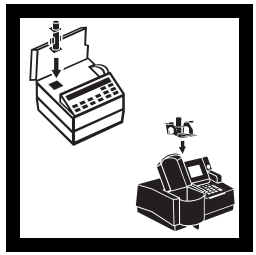
Note: *A 5-mL volumetric or TenSette pipet may be used.*

2. Begin a 20-minute reaction period.

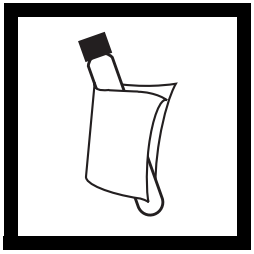
Note: *A pink color will develop if nitrite is present.*



3. Fill an empty Test 'N Tube vial with 5 mL of sample (the blank).



4. Place the COD Vial Adapter into the cell holder.



5. Clean the outside of the vials with a towel.

Note: *Wiping with a damp towel, followed by a dry one, removes fingerprints and other marks.*

6. Place the blank into the vial adapter. Place the cover on the adapter. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 63

DR/2010
Program No. 345
507 nm

DR/4000
Program No. 2630
507 nm

7. Place the prepared sample into the vial adapter. Place the cover on the adapter. Read the mg/L nitrite nitrogen.

Sampling and Storage

Collect samples in clean plastic or glass bottles.

Store at 4 °C (29 °F) or lower if the sample is to be analyzed within 48 hours. Warm to room temperature before analysis.

Accuracy Check

Standard Solution Method
Prepare a nitrite nitrogen standard solution by dissolving 0.493 grams of sodium nitrite, ACS, in 1000 mL of nitrite-free deionized water to give a 100 mg/L nitrite nitrogen (NO_2^-–N) standard solution. This solution is not stable and should be prepared daily. Use a Class A pipet to dilute 1.00 mL of the stock solution to 1000 mL with nitrite-free deionized water to give a

0.10 mg/L (NO_2^-–N) nitrite nitrogen standard solution. Prepare this solution immediately before use.

This solution can also be prepared by diluting 5.00 mL of a 250 mg/L NO_2^-–N stock solution (Cat. No. 23402-49) to 250.0 mL to give a 5.00 mg/L intermediate standard. Dilute 10.00 mL of the 5.0 mg/L intermediate to 500 mL to give a 0.100 mg/L NO_2^-–N standard.

Run the test using the 0.100 mg/L NO_2^-–N standard in place of the sample. Results should be 0.090 to 0.110 mg/L NO_2^-–N.

Interferences

Strong oxidizing and reducing substances interfere.

Cupric and ferrous ions cause low results.

Ferric, mercurous, silver, bismuth, antimonous, lead, auric, chloroplatinate and metavanadate ions interfere by causing precipitation.

Very high levels of nitrate (100 mg/L nitrate as N or more) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.

Summary of Method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Test 'N Tube™ NitriVer® 3 Nitrite Vial	1 vial	50/pkg	26083-45

REQUIRED APPARATUS

COD/TNT Vial Adapter,DR/2010	1	each	44799-00
COD/TNT Vial Adapter, DR/4000	1	each	48189-00
COD/TNT Vial Adapter, DR800	1	each	48464-00
Pipet Filler, safety bulb	1	each	14651-00
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips for 19700-10 TenSette® Pipet	varies	50/pkg	21997-96
Pipet, serological, 5 mL	1	each	532-37
Test 'N Tube™ Vials	1	6/pkg	22758-06
Test 'N Tube™ Vial Caps	1	6/pkg	22411-06
Test Tube Rack	1–3	each	18641-00

OPTIONAL REAGENTS

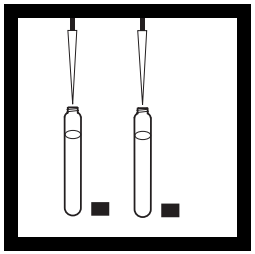
Nitrite Standard Solution, 250 µg/L as NO_2^-–N	500	mL	23402-49
Sodium Nitrite, ACS	454	g	2452-01
Water, deionized	4	L	272-56

OPTIONAL APPARATUS

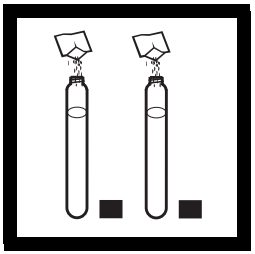
Balance, analytical		each	26103-00
Flask, volumetric, 250 mL		each	14574-46
Flask, volumetric, 500 mL		each	14574-49
Flask, volumetric, 1000 mL		each	547-53
Pipet, serological, 10 mL		each	532-38
Pipet, volumetric, Class A, 1.00 mL		each	14515-35
Pipet, volumetric, Class A, 5.00 mL		each	14515-37
Pipet, volumetric, Class A, 10.00 mL		each	14515-38

NITROGEN, AMMONIA, HR

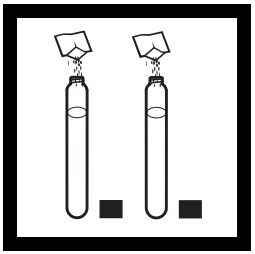
Test 'N Tube Salicylate Method*
Range: 0–50.0 mg/L NH_3–N



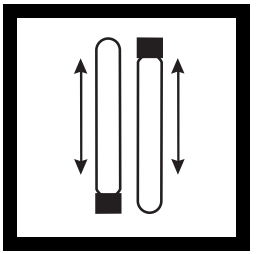
1. Remove the caps from two High Range AmVer Diluent Reagent vials. Add 0.1 mL of sample to one vial (the sample). Add 0.1 mL of ammonia-free water to another (the blank).



2. Add the contents of one Ammonia Salicylate Reagent Powder Pillow for 5 mL sample to each vial.



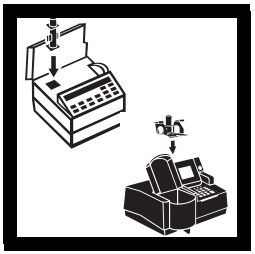
3. Add the contents of one Ammonia Cyanurate Reagent Powder Pillow for 5 mL sample to each vial.



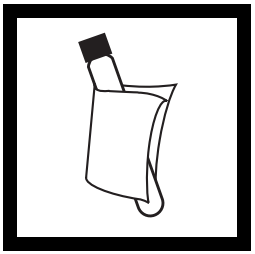
4. Cap the vials tightly and shake thoroughly to dissolve the powder.

Note: *A green color will develop if ammonia is present.*

5. Begin a 20-minute reaction period.

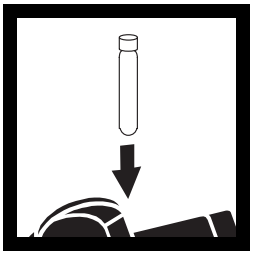


6. Place the COD vial adapter into the cell holder.



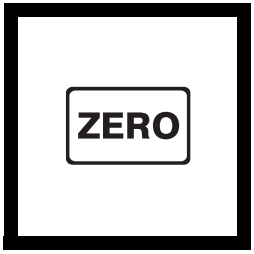
7. Clean the outside of the vial with a towel.

Note: *Wiping with a damp cloth, followed by a dry one, removes fingerprints and other marks.*



8. Place the blank into the vial adapter. Place the cover on the adapter.

* Adapted from *Clin. Chim. Acta*., 14 403 (1966).



9. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 67

DR/2010
Program No. 343
655 nm

DR/4000
Program No. 2465
655 nm

10. Place the prepared sample into the vial adapter. Place the cover on the adapter. Read the mg/L ammonia nitrogen.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl_2 in a one liter sample.* Preserve the sample by reducing the pH to 2 or less with hydrochloric acid (at least 2 mL). Store at 4°C (39 °F) or less. Preserved samples may be stored up to 28 days. Before analysis, warm samples to room temperature and neutralize with 5 N sodium hydroxide. Correct the test result for volume additions.

* Measure chlorine with Hach Water Quality Chlorine Test Strips (27450-50).

Accuracy Check

Standard Solution Method
To check accuracy, use a 10 mg/L Ammonium Nitrogen Standard Solution or use an Ammonium Nitrogen Voluette Ampule Standard, 50 mg/L.

Interferences

The following ions may interfere when present in concentrations exceeding those listed below.

Substance	Concentration
Calcium	50,000 mg/L as $CaCO_3$
Magnesium	300,000 mg/L as $CaCO_3$
Nitrite	600 mg/L as NO_2^-–N
Nitrate	5,000 mg/L as NO_3^-–N
Orthophosphate	5,000 mg/L as PO_4^{3-}-P
Sulfate	6,000 mg/L as SO_4^{2-}

Sulfide will intensify the color. Eliminate sulfide interference as follows:

a. Measure about 350 mL of sample in a 500 mL Erlenmeyer flask.

b. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix.

c. Filter the sample through a folded filter paper.

d. Use the filtered solution in *step 1*.

Iron interferes with the test. Eliminate iron interference as follows:

a. Determine the amount of iron present in the sample following one of the Total Iron procedures.*

* Estimate iron with Hach Water Quality Iron Test Strips (Cat. No. 27453-25).

b. Add the same iron concentration to the ammonia-free water in *step 1*. The interference will then be successfully blanked out.

Acidic or basic samples should be adjusted to approximately pH 7. Use 1 N Sodium Hydroxide Standard Solution for acidic samples and 1 N Hydrochloric Acid Standard Solution for basic samples. Use Hach Water Quality pH Test Strips (Cat. No. 27456-50) to measure pH.

Less common interferences such as hydrazine and glycine will cause intensified colors in the prepared sample. Turbidity and color will give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a green solution.

REQUIRED REAGENTS

Description	Cat. No.
AmVer™ Reagent Set for Nitrogen-Ammonia Determination (50 vials)	26069-45

Includes: (50) AmVer™ Diluent Reagent Vials, (50) Ammonia Salicylate Powder Pillows, (50) Ammonia Cyanurate Powder Pillows. 100 mL deionized water

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
COD/TNT Vial Adapter, DR/2010	1	each	44799-00
COD/TNT Vial Adapter, DR/4000	1	each	48189-00
COD/TNT Vial Adapter, DR/800	1	each	48464-00
Funnel, Micro	1	each	25843-35
Pipet, volumetric, 2.00 mL, Class A	1	each	14515-36
Test Tube Rack	1-3	each	18641-00

OPTIONAL REAGENTS

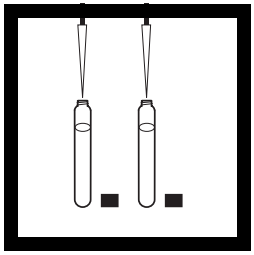
Ammonium Nitrogen Standard Solution, Voluette™ Ampule 50 mg/L NH_3–N, 10 mL	16/pkg	14791-10
Ammonia Nitrogen Standard Solution, 10 mg/L NH_3–N	500 mL	153-49
Hydrochloric Acid, ACS	500 mL	134-49
Sodium Hydroxide Standard Solution, 5.0 N	50 mL	2450-26
Sodium Thiosulfate Standard Solution, 0.1 N	100 mL MDB	323-32
Sulfide Inhibitor Powder Pillows	100/pkg	2418-99
Water, deionized	4 L	272-56
Water Quality Test Strips, total chlorine	50/pkg	27450-50
Water Quality Test Strips, total iron	25/pkg	27453-25
Water Quality Test Strips, pH	50/pkg	27456-50

OPTIONAL APPARATUS

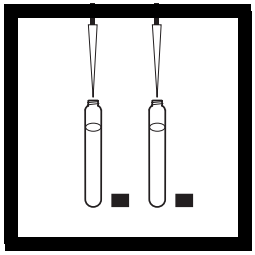
Distillation Apparatus Set, general purpose	each	22653-00
Filter Paper, folded, 12.5 cm	100/pkg	1894-57
Flask, Erlenmeyer, 500 mL	each	505-49
Funnel, analytical	each	1083-68
Heater and Support Apparatus (for distillation), 115 V	each	22744-00
Heater and Support Apparatus (for distillation), 230 V	each	22744-02
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipet, TenSette®, 0-1 mL	each	19700-01
Pipet Tips for 19700-01 TenSette® Pipet	50/pkg	21856-96

NITROGEN, TOTAL INORGANIC

Titanium Trichloride Reduction Method (with centrifuge)
Range: 0–25.0 mg/L N

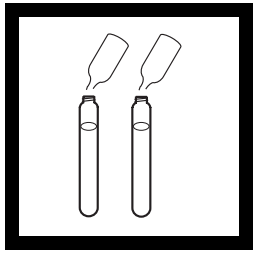


1. Pipet 1 mL of Total Inorganic Nitrogen Pretreatment Base Concentrate into each of two TIN Pretreatment Diluent vials.



2. Pipet 1 mL of sample into one vial (the sample). Pipet 1 mL of deionized water into the other vial (the blank).

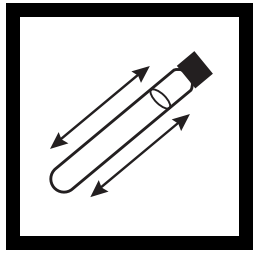
Cap the vials and shake for 30 seconds to mix.



3. Snap the neck off two Total Inorganic Nitrogen Reductant ampules. Pour the contents of one ampule into each vial.

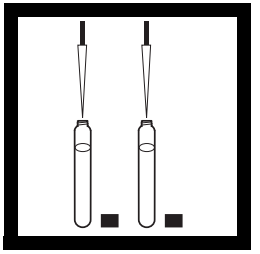
Note: *For safety, wear gloves while breaking the ampules.*

Note: *A black precipitate will form immediately and should remain black. Excessive shaking causes low results.*



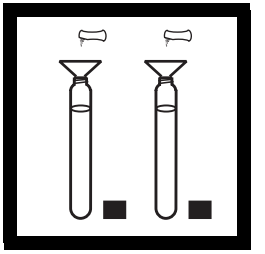
4. Cap the vials. Shake gently for 30 seconds to mix the reagents. Allow the tubes to sit for at least one minute. Centrifuge the tubes for three minutes, or until solids settle to bottom of the tube.

Note: *The solids will settle without use of centrifuge, but can take up to 30 minutes.*

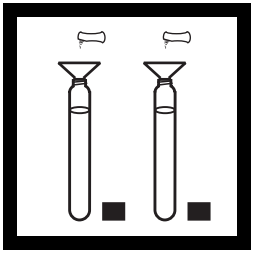


5. Remove the caps from two AmVer Diluent Reagent Test 'N Tubes for Low Range Ammonia Nitrogen. Pipet 2 mL of centrifuged sample into one vial. Add 2 mL of centrifuged blank to another vial. Label the vials appropriately.

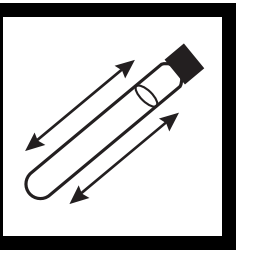
Note: *Pipet carefully to avoid disturbing the sediment.*



6. Add the contents of one Ammonia Salicylate Reagent Powder Pillow to each vial.



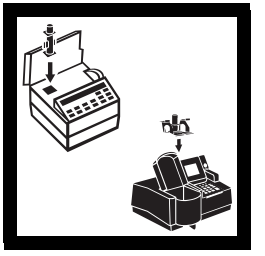
7. Add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each vial.



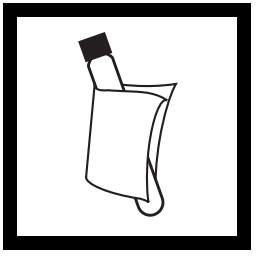
8. Cap the vials tightly and shake thoroughly to dissolve the powder.

Note: *A green color will develop if inorganic nitrogen is present.*

9. Begin a 20-minute reaction period.

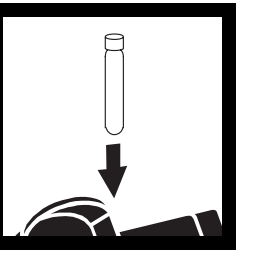


10. During the reaction period, place the COD vial adapter into the cell holder.



11. Clean the outside of the vials with a towel.

Note: *Wiping with a damp cloth followed by a dry one removes fingerprints and other marks.*



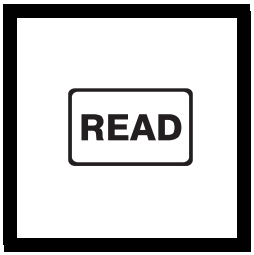
12. When the timer beeps, place the blank in the vial adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

13. Zero the instrument with the blank, using the settings below.

DR/800
Program No. 68

DR/2010
Program No. 346
655 nm

DR/4000
Program No. 2550
655 nm



14. Place the sample in the vial adapter with the Hach logo facing the front of the instrument. Read the mg/L nitrogen (N).

Sampling And Storage

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl_2 in a one liter sample.

Preserve samples by reducing the pH to 2 or less with concentrated hydrochloric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature and neutralize with 5 N Sodium Hydroxide before analysis. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method

a. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.

b. Snap the neck off a fresh High Range Nitrate Nitrogen Voluette Ampule Standard, 500 mg/L NO_3^-–N.

c. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard, respectively, to the three 25-mL mixing cylinders. Mix thoroughly.

d. Analyze each sample as described in the procedure; use a 1-mL aliquot of the prepared sample in each test. The nitrogen concentration should increase 1.8 to 1.9 mg/L for each 0.1 mL of standard added.

Standard Solution Method

To check accuracy, use a 10.0 mg/L Nitrate Nitrogen Standard Solution listed under Optional Reagents. Or, this can be prepared by pipetting 5-mL of solution from a Voluette Ampule Standard for High Range Nitrate Nitrogen, 500 mg/L NO_3^-–N, into a 250-mL volumetric flask. Dilute to 250 mL with deionized water. Substitute this standard for the sample and perform the test as described. The mg/L NO_3^-–N reading should be 9–10 mg/L N.

Application Information

The total inorganic nitrogen test provides an estimate of the total nitrite, nitrate, and ammonia nitrogen load in water or wastewater samples. This test is most applicable for monitoring an industrial process stream or a wastewater treatment stream where it is important to track the inorganic nitrogen load as it passes through the treatment process. The test exhibits different recoveries of each of the three nitrogen species (NH_3^-–N, NO_3^-–N, NO_2^-–N). This test is not recommended for quantifying only one of the three species. In that case, use a specific procedure for each particular analyte.

Interferences

The following ions may interfere when present in concentrations exceeding those listed below:

Species	Level	Effect
Calcium	1000 mg/L as $CaCO_3$	Positive
Manganese (IV)	3 mg/L	Negative
Magnesium	1000 mg/L as $CaCO_3$	Positive
Sulfide	3 mg/L	Negative
Sulfate	250 mg/L	Negative

The following do not interfere below the levels listed:

Species	Level
Al^{3+}	8 mg/L
Ba^{2+}	40 mg/L
Cu^{2+}	40 mg/L
Fe^{3+}	8 mg/L
Zn^{2+}	80 mg/L
F^-	40 mg/L
PO_4^{3-}-P	8 mg/L
SiO_2	80 mg/L
EDTA	80 mg/L

Summary of Method

Titanium (III) ions reduce nitrate and nitrite to ammonia in a basic environment. After centrifugation to remove solids, the ammonia is combined with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution.

REQUIRED REAGENTS

Description	Cat. No.
Total Inorganic Nitrogen Pretreatment Reagent Set ($TiCl_3$ Reduction)	26049-45
Includes: 50 Total Inorganic Nitrogen Pretreatment Diluent Vials, TIN Pretreatment Base Concentrate, and 50 ampules of Total Inorganic Nitrogen Reductant	
AmVer™ Reagent Set for LR Nitrogen-Ammonia Determination	26045-45
Includes: 50 AmVer™ Diluent Reagent Low Range Vials, 50 Ammonia Salicylate Powder Pillows, and 50 Ammonia Cyanurate Powder Pillows	

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
Centrifuge, 115 V	1	each	26765-00
Centrifuge, 230 V	1	each	26765-02
COD/TNT Vial Adapter, DR/2010	1	each	44799-00
COD/TNT Vial Adapter, DR/4000	1	each	48189-00
COD/TNT Vial Adapter, DR800	1	each	48464-00
Funnel, micro	1	each	25843-35
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips for 19700-01 TenSette® Pipet	2	50/pkg	21856-96
Safety Bulb	1	each	14651-00
Test Tube Rack	1	each	18641-00

OPTIONAL REAGENTS

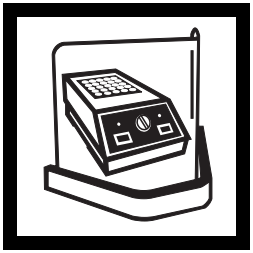
Hydrochloric Acid, ACS	500 mL	134-49
Nitrate Nitrogen Standard Solution, 10 mg/L NO_3^-–N	500 mL	307-49
Nitrate Nitrogen Standard Solution, Voluette™ Ampule, 500 mg/L NO_3^-–N	16/pkg	14260-10
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	2450-26
Sodium Thiosulfate Standard Solution, 0.1 N	100 mL MDB	323-32
Water, deionized	4 L	272-56

OPTIONAL APPARATUS

Ampule Breaker Kit	each	21968-00
Cylinder, graduated, mixing	each	20886-40
Flask, volumetric, Class A, 250.0 mL	each	14574-41
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipette, volumetric, Class A, 5.00 mL	each	14515-37

NITROGEN, TOTAL*

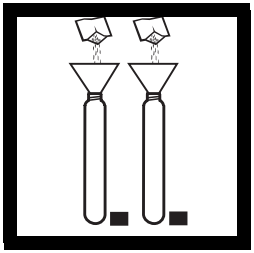
Test 'N Tube Persulfate Method
Range: 0–25 mg/L N



1. Turn on the COD Reactor. Heat to 103-106 °C (optimum temperature is 105 °C). Place the plastic shield in front of the reactor.

Note: *Ensure safety devices are in place to protect the analyst from splattering should leakage occur.*

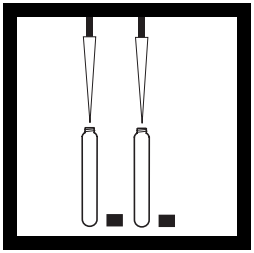
Note: *For proof of accuracy, run a 20 mg/L NH_3–N standard through digestion and analysis.*



2. Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two Total Nitrogen Hydroxide Reagent vials.

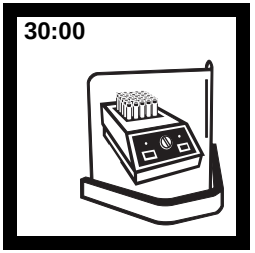
Note: *Wipe off any reagent that may get on the lid or the tube threads.*

Note: *One reagent blank is sufficient for each set of samples.*



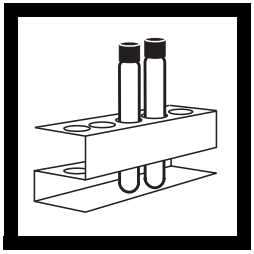
3. Add 2 mL of sample to one vial. Add 2 mL of organic-free water to another vial (the reagent blank). Cap both vials and shake vigorously (at least 30 seconds). The persulfate reagent may not dissolve completely after mixing.

Note: *Alternate water must be free of all nitrogen-containing species.*



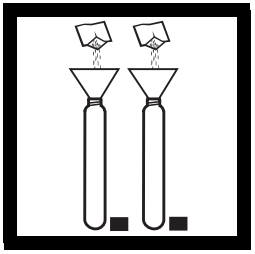
4. Place the vials in the COD Reactor. Heat for 30 minutes.

* A 10–150 mg/L N range method is now available in a reagent set (Cat. No. 27141-00). Please call Customer Service for more information.



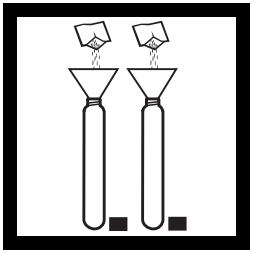
5. Using finger cots or gloves, remove the hot vials from the reactor and allow to cool to room temperature.

Note: *It is very important to remove the vials from the COD Reactor after exactly 30 minutes.*



6. Remove the caps from the cooled digested vials and add the contents of one TN Reagent A Powder Pillow to each vial. Cap vials and shake for 15 seconds.

7. Begin a three-minute reaction period.

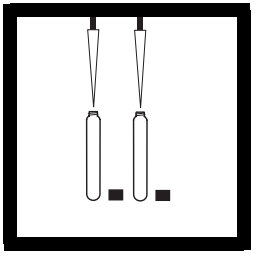


8. After the timer beeps, remove the caps from the cooled vials and add one TN Reagent B Powder Pillow to each vial. Cap vials and shake for 15 seconds.

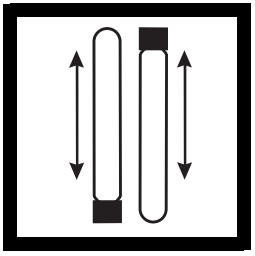
9. Begin a two-minute reaction period.

Note: *The reagent will not completely dissolve.*

Note: *The solution will begin to turn yellow.*



10. After the timer beeps, remove the caps from two TN Reagent C Vials and add 2 mL of digested, treated sample to one vial. Add 2 mL of the digested, treated reagent blank to the second TN Reagent C Vial.

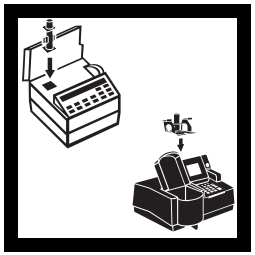


11. Cap vials and invert 10 times to mix. Use slow, deliberate inversions for complete recovery. The vials will be warm.

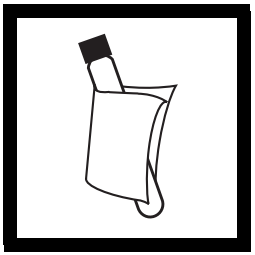
Note: *Hold the vial vertical with the cap up. Invert the vial and wait for all of the solution to flow to the cap end. Return the vial to the upright position and wait for all of the solution to flow to the vial bottom. This is one inversion (10 inversions = 30 seconds)*

12. Begin a five-minute reaction period. Do not invert the vials again.

Note: *The yellow color will intensify.*



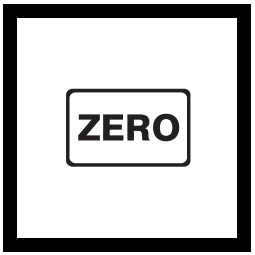
13. Place the COD/TNT vial adapter in the cell holder.



14. When the timer beeps, wipe the vials.

Note: *Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.*

Note: *The reagent blank is stable when stored in the dark; see Blanks for Colorimetric Measurement following this procedure.*



15. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 58

DR/2010
Program No. 350
410 nm

DR/4000
Program No. 2558
410 nm

16. Place the sample in the cell holder. Read the mg/L nitrogen.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.

Preserve the sample by reducing the pH to 2 or less with concentrated sulfuric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature and neutralize with 5 N sodium hydroxide before analysis. Correct the test result for volume additions.

Accuracy Check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy Hach offers a set of 3 Primary Standards for Kjeldahl Nitrogen.

1. Prepare one or more of the following 3 solutions. Each preparation is for an equivalent 25 mg/L N standard.

a. Weigh 0.3379 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a Class A 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.

b. Weigh 0.4416 g of Glycine p-Toluenesulfonate. Dissolve in a Class A 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.

c. Weigh 0.5274 g of Nicotinic p-Toluenesulfonate. Dissolve in a Class A 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.

2. Analyze each of these solutions using the test procedure. Calculate the percent recovery for each using this formula:

$$\%\ \text{recovery} = \frac{\text{measured concentration}}{25} \times 100$$

The percent recovery should be:

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Hach analysts have found Ammonia-PTSA to be the most difficult to digest.

Other compounds may yield different percent recoveries.

Standard Solution Method

For proof of accuracy, substitute 2 mL of a 20 mg/L ammonia nitrogen standard solution for the sample in the procedure. To prepare a 20-mg/L standard, use a 20-mL Class A pipet to

transfer 20 mL of a 100-mg/L Ammonia Nitrogen Standard to a 100-mL Class A volumetric flask. Dilute to the line with organic-free water. A single analyst should obtain less than 5% variation on replicates.

Standard Additions Method

a. Fill three, 25-mL graduated mixing cylinders with 25 mL of sample.

b. Snap the neck off an Ammonia Nitrogen Voluette Ampule Standard Solution, 160 mg/L as NH_3-N.

c. Use the TenSette Pipet to add 0.3 mL, 0.6 mL, and 0.9 mL of standard, respectively, to the 3 mixing cylinders.

d. Stopper each cylinder and mix thoroughly.

e. Add 2 mL of each prepared solution, respectively, to three Total Nitrogen Hydroxide Reagent Vials.

f. Analyze each standard addition sample as described in the procedure. The nitrogen concentration should increase by approximately 1.9, 3.8 and 5.6 mg/L N, respectively.

g. If these increases do not occur, an interference is likely

Blanks For Colorimetric Determination

The reagent blank may be used repeatedly for measurements using the same lots of reagents. Store the reagent blank in the dark at room temperature (18–25 °C) for a maximum of seven days. If a small amount of white floc appears prior to the end of one week, discard the reagent blank and prepare a new one.

Interferences

The substances in the following table have been tested and found **not** to interfere up to the indicated levels (in mg/L):

Substance	Maximum Level Tested (mg/L)	Substance	Maximum Level Tested (mg/L)
Barium	2.6	Organic Carbon	150
Calcium	300	pH	13 pH units
Chromium (3+)	0.5	Phosphorus	100
Iron	2	Silica	150
Lead	6.6 ppb	Silver	0.9
Magnesium	500	Tin	1.5

Interfering substances that resulted in a concentration change of ±10%:

Substance	Level and Effect
Bromide	>60ppm; positive interference
Chloride	>1000 ppm; positive interference*

* Measure chloride with Hach Water Quality Test Strips, High Range Chloride (Cat. No. 27513-40).

Hach chemists tested this chemistry on standard nitrogen solutions prepared from the following compounds and obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Urea
- Glycine

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in ≥95% recovery.

Summary of Method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

REQUIRED REAGENTS

Description	Cat. No.
Test 'N Tube Total Nitrogen Reagent Set (50 vials)	26722-45

Includes:
(50) Total Nitrogen Hydroxide Reagent Vials,
(50) Total Nitrogen Persulfate Reagent Powder Pillows,
(50) TN Reagent A Powder Pillows, (50) TN Reagent B Powder Pillows, and (50) TN Reagent C Vials.

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
COD Reactor, 115/230 V, North American Plug	1	each	45600-00
COD Reactor, 230 V, European Plug	1	each	45600-02
COD/TNT Vial Adapter, DR/2010	1	each	44799-00
COD/TNT Vial Adapter, DR/4000	1	each	48189-00
COD/TNT Vial Adapter, DR/800	1	each	48464-00
Funnel, Micro	3	each	25843-35
Safety Shield	1	each	50030-00
Test Tube Cooling Rack	1-3	each	18641-00

OPTIONAL REAGENTS

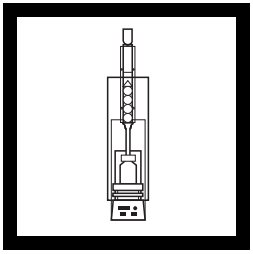
Description	Unit	Cat. No.
Nitrogen, Ammonia, 100 mg/L NH_3-N	500 mL	24065-49
Nitrogen, Ammonia, Voluette™ Ampule, 160 mg/L NH_3-N, 10 mL	16/pkg	21091-10
Sulfuric Acid, ACS	500 mL	979-49
Primary Standards for Kjeldahl Nitrogen	set of 3	22778-00
Sodium Hydroxide Standard Solution, 5.0 N	59 mL MDB	2450-26
Water, Organic-free	500 mL	26415-49
Water Quality Test Strips, high range chloride	40/pkg	27513-40

OPTIONAL APPARATUS

Balance, analytical, 110/220V	each	22310-00
Cylinder, graduated, mixing, 25 mL	each	20886-40
Flask, volumetric, Class A, 1000 mL (3 required)	each	14574-53
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipet, TenSette®, 0.1 to 1.0 mL (includes 50 tips)	each	19700-01
Pipet Tips for 19700-01 TenSette® Pipet	50/pkg	21856-96

NITROGEN, TOTAL KJELDAHL

Nessler Method*
Range: Liquids: 0.5–22500 mg/L; Solids: 30–225000 mg/kg

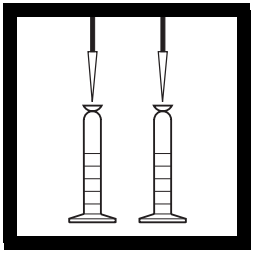


1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *SECTION 3*.

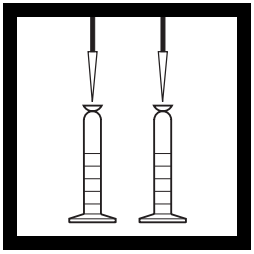
Note: *Sensitivity to wavelength setting and reagent lot variation necessitate user calibration for best results.*

Note: *For best results, digest and analyze a reagent blank using deionized water as the sample. Subtract the blank value from the displayed sample value.*

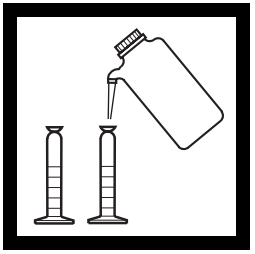
Note: *If sample cannot be analyzed shortly after collection, see* Sampling and Storage *following these steps.*



2. Use the analysis volume in the same table. Pipet the sample and blank analysis volume into separate 25-mL mixing cylinders.

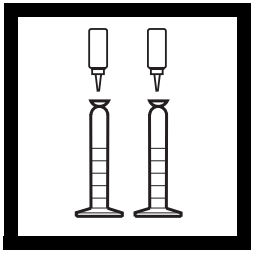


3. Add one drop TKN Indicator to each cylinder. Add 8.0 N KOH dropwise to each cylinder, mixing after each addition. Continue until the first apparent blue color is visible.



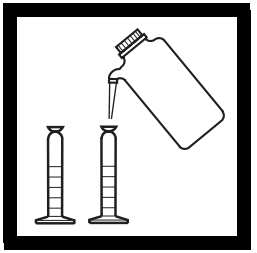
4. Fill both mixing cylinders to the 20-mL mark with deionized water.

* Adapted from: Hach et al., *Journal of Association of Official Analytical Chemists*, 70 (5) 783-787 (1987); Hach et al., *Journal of Agricultural and Food Chemistry*, 33 (6) 1117-1123 (1985); *Standard Methods for the Examination of Wastewater.*

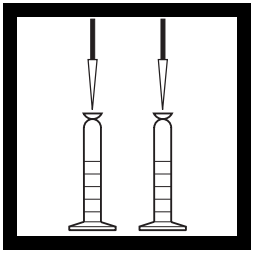


5. Add 3 drops of Mineral Stabilizer to each cylinder. Invert several times to mix. Add 3 drops of Polyvinyl Alcohol Dispersing Agent to each cylinder. Invert several times to mix.

Note: *Hold the dropping bottles upright while dispensing.*



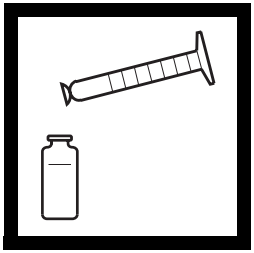
6. Fill both cylinders to the 25-mL mark with deionized water.



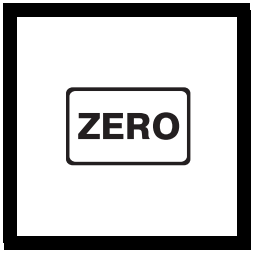
7. Pipet 1 mL of Nesslers Reagent to each cylinder. Stopper, and invert repeatedly. The solution should not be hazy

Note: *Any haze (or turbidity) will cause incorrect results.*

8. Begin a two-minute reaction period.



9. After the reaction period, pour the contents of each cylinder into separate 25-mL sample cells.

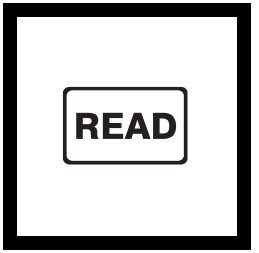


10. Zero the instrument with the blank, using the settings below.

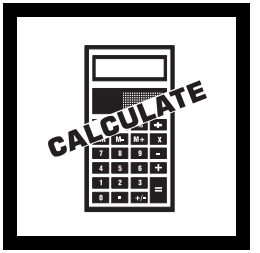
DR/800s
Program No. 65

DR/2010
Program No. 399 or user-program number
460 nm

DR/4000
Program No. 2410 or user-program number
460 nm



11. Place the sample in the cell holder. Read the mg/L TKN.



12. Use the equation below the Nitrogen TKN Sample and Analysis Volume Tables to calculate the true TKN concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Note: *The calculation uses 75; the calibration is done with a 25-mL digestion volume and a 3 mL analysis volume.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Nitrogen Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.5–28	40.0*	10.0*	25 mL
2–112	20.0*	5.00*	25 mL
11–560	10.0*	2.00*	25 mL
45–2250	5.00*	1.00*	25 mL
425–22500	1.00*	0.50*	25 mL

Solids

Expected Nitrogen Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
30–2250	0.500*	10.0*	25 mL
75–5620	0.400*	5.00*	25 mL
250–18750	0.300*	2.00*	25 mL
750–56250	0.200*	1.00*	25 mL
3000–225000	0.100*	0.50*	25 mL

* The 75 in the formula was derived from a multiplication factor using the theoretical volumes of 25 mL and 3 mL.

Calculation For Final Concentration:

$$\frac{A \times 75}{B \times C} = \text{mg/L or mg/kg TKN}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in a cleaned glass or plastic container. Adjust the pH to 2 or less with sulfuric acid (about 2 mL per liter) and cool to 4 °C. Preserved samples can be stored up to 28 days.

Accuracy Check

Standard Additions Method

a. Perform the TKN method and note the analysis volume used and the mg/L N of the sample.

b. Pipet the same analysis volume into three 25-mL graduated mixing cylinders.

c. Snap the neck of a 150-mg/L Ammonia Voluette Ampule Standard.

d. Use a TenSette pipet to add 0.1, 0.2 and 0.3 ml of standard, respectively, to the cylinders. Dilute to 25 mL and mix well.

e. Perform the TKN methods beginning with step 5. The nitrogen level should increase 20 mg/L for each 0.1 ml of standard added (compared to step a).

f. If these increases do not occur, an interference is likely.

Standard Solution Method

Add one drop TKN indicator to each of two 25-mL mixing cylinders. Fill one cylinder with deionized water to the 20-mL mark. Fill the other cylinder to the 20-mL mark with a 1.0 mg/L NH_3-N solution. Add 3 drops Mineral Stabilizer to each cylinder. Invert to mix. Add 3 drops Polyvinyl Alcohol Dispersing Agent to each cylinder. Invert to mix. Perform the Total Kjeldahl Nitrogen procedure starting with *step* 6. This display should show 26-27 mg/L TKN in *step 11*.

Kjeldahl Nitrogen Standard Method

This checks the efficiency of the digestion and colorimetric test. There are standards available for doing this test. For a complete procedure, see the *Accuracy Check* in *Section 3.3.8* on page *27*.

Summary of Method

"Total Kjeldahl Nitrogen" (also called crude protein) refers to the combination of ammonia nitrogen and organic nitrogen composed of trinegative nitrogen. These compounds are converted into ammonium salts by the action of sulfuric acid and hydrogen peroxide. The ammonia salts plus any ammonia present are then analyzed by a modified Nessler method test. The Mineral Stabilizer complexes calcium and magnesium. The Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonium ions. A yellow color forms, proportional to the ammonia concentration.

REQUIRED REAGENTS

Description	Cat. No.
TKN Nitrogen Reagent Set	24953-00
Includes: (1) 282-32, (1) 979-49, (1) 21194-49, 1) 21196-49, (1) 22519-26, (1) 23144-26, (1) 23765-26, (1) 23766-26	

Description	Quantity Required Per Test	Unit	Cat. No.
Hydrogen Peroxide, 50%	20 mL	490 mL	21196-49
Mineral Stabilizer	6 drops	50 mL SCDB	23766-26
Nesslers Reagent	2 mL	500 mL	21194-49
Polyvinyl Alcohol Dispersing Agent	6 drops	50 mL SCDB	23765-26
Potassium Hydroxide Standard Solution, 8.0 N	varies	100 mL MDB	282-32
Potassium Hydroxide Standard Solution, 1.0 N	varies	100 mL MDB	23144-26
Sulfuric Acid, ACS	6 mL	500 mL	979-49
TKN Indicator Solution	2 drops	50 mL SCDB	22519-26
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Boiling Chips, silicon carbide	2–3	500 g	20557-34
Cylinder, graduated, mixing, tall-form, 25 mL	2	each	21190-40
Pipet, TenSette® 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	2	50/pkg	21856-96
Safety Shield, for Digesdahl®	1	each	50040-00

Select one based on available voltage:

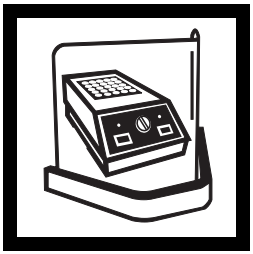
Digesdahl® digestion apparatus, 115 Vac	1	each	23130-20
Digesdahl® digestion apparatus, 230 Vac	1	each	23130-21

Select one or more based an sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

NITROGEN, TOTAL, High Range

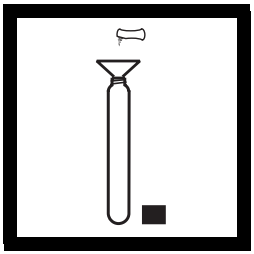
Persulfate Test 'N Tube Digestion Method
Range: 0 to 150 mg/L N



1. Turn on the COD Reactor. Heat to 103-106 °C (optimum temperature is 105 °C). Place the plastic shield in front of the reactor.

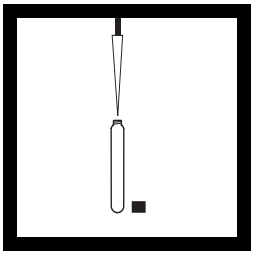
Note: *Ensure safety devices are in place to protect the analyst should splattering and leakage occur.*

Note: *For proof of accuracy, run a 125 mg/L NH_3-N standard through digestion and analysis.*



2. Prepare a reagent blank: Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to one HR Total Nitrogen Hydroxide Digestion Vial.

Note: *Wipe off any reagent that gets on the lid or the tube threads.*



3. Add 0.5 mL of organic-free water to the vial. Cap the vial and shake vigorously for about 30 seconds.

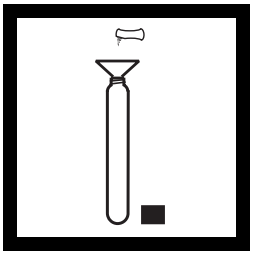
Process this reagent blank exactly the same as the sample, including digestion and color finish. Proceed to *step 6*.

Note: *Alternate water must be free of all nitrogen-containing species.*

Note: *The persulfate reagent may not dissolve completely after shaking.*

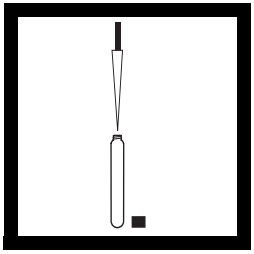
Note: *One reagent blank is sufficient for each set of samples using the same lots of reagents.*

Note: *The reagent blank is stable for as long as seven days when stored in the dark; see* Blanks for Colorimetric Measurement *following this procedure.*



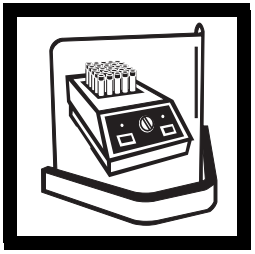
4. Prepare a sample: Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to one HR Total Nitrogen Hydroxide Digestion Vial.

Note: *Wipe off any reagent that gets on the lid or the tube threads.*

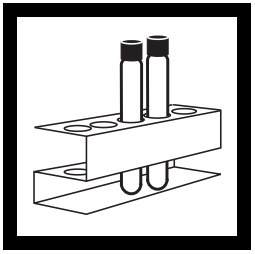


5. Add 0.5 mL of sample to the vial. Cap the vial and shake vigorously for about 30 seconds.

Note: *The persulfate reagent may not dissolve completely after shaking.*

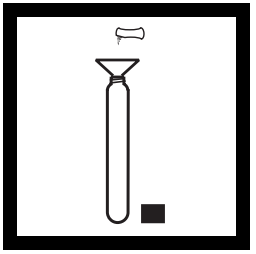


6. Place the vials in the COD Reactor. Heat for 30 minutes.



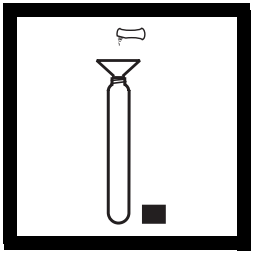
7. Using finger cots or gloves, remove the hot vials from the reactor and allow to cool to room temperature.

Note: *It is very important to remove the vials from the COD Reactor after exactly 30 minutes.*



8. Add the contents of one Total Nitrogen Reagent A Powder Pillow to the vial containing the digested blank or sample. Cap the vial and shake for 15 seconds.

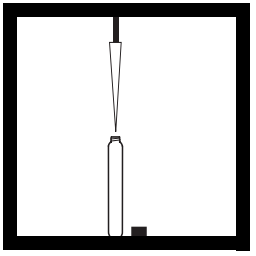
9. After shaking, begin a three-minute reaction period.



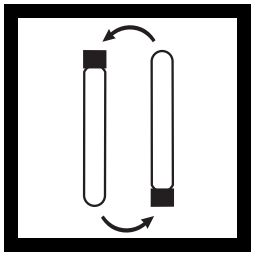
10. After the timer beeps, add one Total Nitrogen Reagent B Powder Pillow to the vial. Cap the vial and shake for 15 seconds.

11. After shaking, begin a two-minute reaction period.

Note: *The reagent will not dissolve completely. The solution will begin to turn yellow.*



12. After the timer beeps, remove the cap from the Total Nitrogen Reagent C Vial. Add 2 mL of digested, treated sample (or reagent blank) to the vial. The vial will be warm.

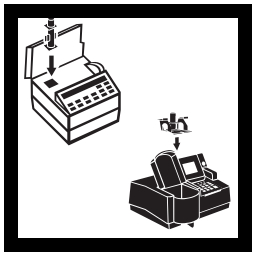


13. Cap vial and invert slowly 10 times to mix. The vial will be warm.

Note: *Proper mixing is important for complete recovery. Hold the vial vertical with the cap up. Invert the vial and wait for all of the solution to flow to the cap end. Pause. Return the vial to the upright position and wait for all of the solution to flow to the vial bottom. This is one inversion (10 inversions = 30 seconds)*

14. Begin a five minute reaction period. Do not invert the vial again.

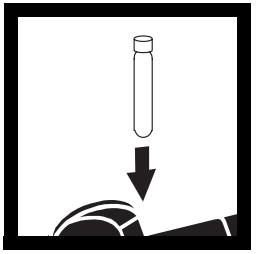
Note: *The yellow color will intensify.*



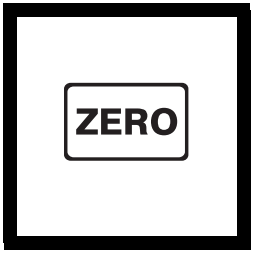
15. Place the COD Vial adapter into the cell holder.

16. When the timer beeps, wipe the outside of the Total Nitrogen Reagent C vial containing the reagent blank. Place the vial into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

Note: *Wipe with a damp towel, followed by a dry one, to remove fingerprints or other marks.*



17. Place the sample vial in the adapter. Place the cover on the adapter.

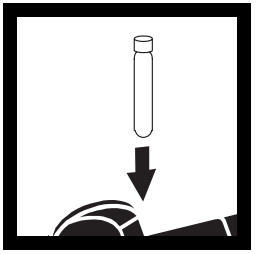


18. Zero the instrument with the vial, using the settings below.

DR/800s
Program No.69

DR/2010
Program No. 395
410 nm

DR/4000
Program No. 2559
410 nm



19. Place the sample vial into the adapter. Read the mg/L nitrogen.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.

Preserve the sample by reducing the pH to 2 or less with concentrated sulfuric acid (at least 2 mL per liter). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Warm the samples to room temperature and neutralize with 5 N sodium hydroxide before analysis. Correct the test result for volume additions.

Accuracy Check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy, Hach offers a set of three Primary Standards for Kjeldahl Nitrogen.

1. Prepare one or more of the following solutions. Each preparation is for an equivalent 120 mg/L N standard. Use water that is free of all organic and nitrogen-containing species.

   a. Weigh 1.6208 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a Class A 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.

   b. Weigh 2.1179 g of Glycine p-Toluenesulfonate. Dissolve in a Class A 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.

   c. Weigh 2.5295 g of Nicotinic p-Toluenesulfonate. Dissolve in a Class A 1000-mL volumetric flask with deionized water. Add deionized water to the1000-mL mark.

2. Analyze each of these solutions using the test procedure. Calculate the percent recovery for each using this formula:

$$\%\ \text{recovery} = \frac{\text{measured concentration}}{120} \times 100$$

The percent recovery should be:

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Hach analysts have found Ammonia-PTSA to be the most difficult to digest. Other compounds may yield different percent recoveries.

Standard Solution Method

For proof of accuracy, substitute 0.5 mL of a 125 mg/L ammonia nitrogen standard solution for the sample in the procedure. To prepare a 125-mg/L N standard, use a 25-mL Class A pipet to transfer 25.00 mL of a 1000-mg/L Ammonia Nitrogen Standard (see *OPTIONAL REAGENTS* on page *263*.) to a 200-mL Class A volumetric flask. Dilute to the line with organic-free water.

Standard Additions Method

a. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.

b. Snap the neck off an Ammonia Nitrogen Voluette Ampule Standard Solution, 1000 mg/L as NH_3–N.

c. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to the three mixing cylinders.

d. Stopper each cylinder and mix thoroughly.

e. Add 0.5 mL of each prepared solution, respectively, to three HR Total Nitrogen Hydroxide Digestion Vials.

f. Analyze each standard addition sample as described in the procedure. The nitrogen concentration should increase by approximately 4 mg/L N for each 0.1 mL of standard added.

g. If these increases do not occur, an interference is likely.

Blanks for Colorimetric Measurement

The reagent blank may be used repeatedly for measurements using the same lots of reagents. Store the reagent blank in the dark at room temperature (18–25 °C) for a maximum of seven days. If a small amount of white floc appears prior to the end of one week, discard the reagent blank and prepare a new one.

Interferences

The substances in the following table have been tested and found **not** to interfere up to the indicated levels:

Substance	Maximum Level Tested (mg/L)	Substance	Maximum Level Tested (mg/L)
Barium	10.4	Organic Carbon	600
Calcium	1200	pH	13 pH units
Chromium^{3+}	2	Phosphorus	400
Iron	8	Silica	600
Lead	26.4 ppb	Silver	3.6
Magnesium	2000	Tin	6.0

Interfering substances that resulted in a concentration change of ±10%:

Substance	Level and Effect
Bromide	> 240 ppm; positive interference
Chloride	≥ 3000 ppm; positive interference

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate reagent. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Summary of Method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

REQUIRED REAGENTS

Description	Cat. No.
Test 'N Tube™ HR Total Nitrogen Reagent Set (50 vials) ..... Includes: (1) 26718-46, (1) 26719-46, (1) 26720-46, (50) Hydroxide Digestion Vials,* (50) Acid Solution Vials*	27141-00

Description	Quantity Required Per Test	Unit	Cat. No.
HR Total Nitrogen Hydroxide Digestion Vials	1 vial	50/pkg	*
Total Nitrogen Persulfate Reagent Powder Pillows	1 pillow	50/pkg	26718-46
Total Nitrogen Reagent A, Bisulfite Powder Pillows	1 pillow	50/pkg	26719-46
Total Nitrogen Reagent B, Indicator Powder Pillows	1 pillow	50/pkg	26720-46
Total Nitrogen Reagent C Vials, Acid Solution	1 vial	50/pkg	*

REQUIRED APPARATUS

COD Reactor, 115/230 V, North American Plug	1	each	45600-00
COD Reactor, 230 V, European Plug	1	each	45600-02
COD Vial Adapter, DR/2010	1	each	44799-00
COD Vial Adapter, DR/4000	1	each	48189-00
COD Vial Adapter, DR/800	1	each	48464-00
Funnel, Micro	3	each	25843-35
Pipet, TenSette®, 0.1–1.0 mL	1	each	19700-01
Pipet Tips for 19700-01 Tensette® Pipet	1	50/pkg	21997-96
Safety Shield	1	each	23810-00
Test Tube Cooling Rack	1-3	each	18641-00

* These Items are not sold separately. Please order the complete set (Cat. No. 27141-00) as a replacement.

OPTIONAL REAGENTS

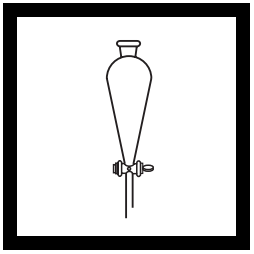
Description	Unit	Cat. No.
Nitrogen, Ammonia, 1000 mg/L NH_3–N	1 L	23541-53
Nitrogen, Ammonia, Voluette™ Ampule, 1000 mg/L NH_3–N, 10 mL	16/pkg	23541-10
Primary Standards for Kjeldahl Nitrogen	set of 3	22778-00
Sodium Hydroxide Standard Solution, 5.0 N	59 mL MDB	2450-26
Sulfuric Acid, ACS	500 mL	979-49
Water, organic-free	500 mL	26415-49

OPTIONAL APPARATUS

Ampule Breaker Kit,	each	21968-00
Balance, analytical, 115 Vac	each	26103-00
Balance, analytical, 230 Vac	each	26103-02
Cots, finger	2/pkg	14647-02
Cylinder, graduated, mixing, 25 mL, 3 needed	each	20886-40
Flask, Volumetric, Class A, 200 mL	each	14574-45
Flask, volumetric, Class A, 1000 mL, 3 needed	each	14574-53
Pipet, Volumetric, Class A, 25 mL	each	14515-40

OIL AND GREASE

Hexane Extractable Gravimetric Method*
Range: 15–3000 mg/L HEM and SGT-HEM



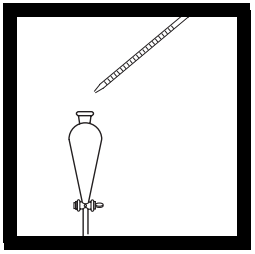
1. Collect 350 mL of sample in a clean 500- mL separatory funnel.

Note: *Do not pre-rinse the collecting vessel with sample or results may be increased.*

Note: *If the sample is not collected in the separatory funnel, set the empty container and lid aside for use in step 4.*

Note: *The sample must be at room temperature before analyzing.*

Note: *Determine a blank value (350 mL of distilled or deionized water) with each new lot of reagents. If the blank result is greater than 5 mg, resolve the source of error or remove interferences before performing this procedure.*

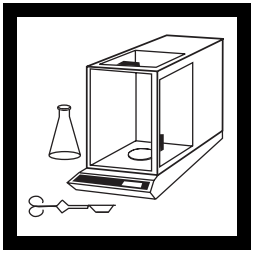


2. Using a pipette and pipette filler, add 4 mL of 1:1 Hydrochloric Acid solution to the separatory funnel. Mix well. The pH must be less than or equal to 2.

Note: *Check sample pH after acid addition by dipping a glass rod into the sample and allowing a few drops to touch the pH paper. Do not dip the pH paper into the sample. Rinse the glass rod with a small portion of hexane back into the separatory funnel to remove any grease/oil on the rod.*

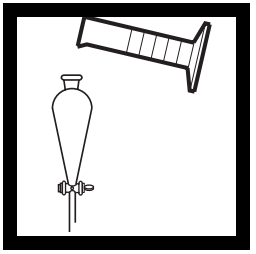
Note: *A pH of ≤2 is required to hydrolyze some oils and greases. A pH of greater than 2 dissolves the sodium sulfate used in step 8 and causes an interference.*

Note: *Use the equivalent amount of acid to determine the blank and all samples from each sampling source.*



3. Using an analytical balance, weigh a previously dried and cleaned 125-mL distillation flask containing 3–5 boiling chips to the nearest 0.1 mg. Record the weight of the flask.

Note: *If determining both the HEM and the SGT-HEM, clean and dry two distillation flasks (one for each procedure) in advance. A third clean, dry flask may be needed. See the note under step 12.*

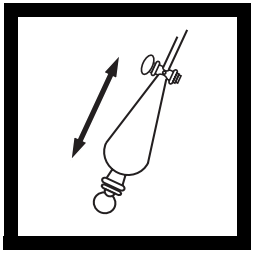


4. Add 20 mL of n-hexane to the separatory funnel.

Note: *Spilled reagent will affect test accuracy and is hazardous to skin and other materials.*

Note: *If the sample was collected in a separate container or if repeating this step, rinse the collecting vessel/volumetric flask which contained the sample/water layer with the 20 mL of n-hexane, then add the 20-mL n-hexane rinse to the separatory funnel.*

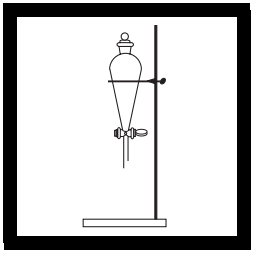
* Equivalent to USEPA Method 1664.



5. Stopper the funnel. Invert the funnel and release the gases through the stopcock. Then, vigorously shake the funnel for two minutes.

Note: *To release gases from the separatory funnel, invert it and shake it once very hard (support the stopper with your hand). Under a hood, point the delivery tube in a safe direction and SLOWLY open the stopcock to release any gas. Close the stopcock. Repeat the venting until you no longer hear the release of gases.*

Note: *Do not count the venting time as part of the two-minute shaking time. Shaking for less than two minutes may decrease the results.*

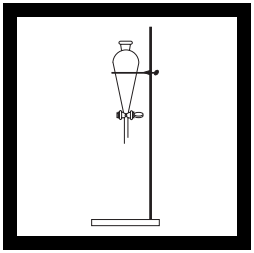


6. Let the funnel stand undisturbed for **at least** 10 minutes to ensure separation of the lower water layer and the upper solvent layer.

Note: *The solvent layer may be brown if a colored oil is present.*

Note: *If an emulsion forms, see Interferences following this procedure. An emulsion is a bubbly layer between the aqueous and solvent layer.*

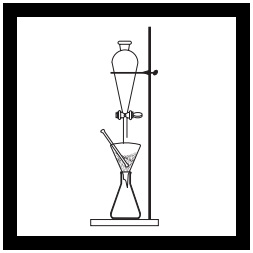
Note: *If you repeat this step the third time and the water layer is cloudy, allow the separatory funnel to stand undisturbed for 20 minutes for better separation of the water and solvent layers.*



7. **SLOWLY** drain the lower water layer from the separatory flask into the original sample container or 500-mL volumetric flask. This should take about 3–4 minutes. Save the water layer for *step 9*.

Note: *To ensure water is not transferred in step 8, allow several drops of solvent layer to drain into the water layer until the solvent layer is visible on top of the water.*

Note: *If the water layer drains too quickly, excess water will be present in the solvent layer. This causes sodium sulfate and water interference.*

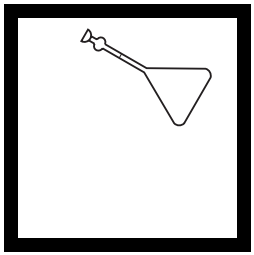


8. Drip-drain the solvent layer into the pre-weighed boiling flask through a funnel containing filter paper and 10 g anhydrous sodium sulfate. Gently stir the sodium sulfate with a glass stirring rod while the solvent layer is draining. Be careful not to rip the filter paper.

Note: *Use the same filter, funnel, and sodium sulfate when repeating this step for the second and third extractions. Remove large, hard sodium sulfate chunks between extractions to reduce sodium sulfate contamination.*

Note: *To set up the filtering funnel, put the glass funnel in the neck of the distillation flask. Place a folded 12.5 cm filter paper in the funnel. Add 10 grams of anhydrous sodium sulfate to the filter paper. Rinse the sodium sulfate with a small amount of the hexane. Discard the hexane properly.*

Note: *Do not use any plastic tubing to transfer the solvent.*



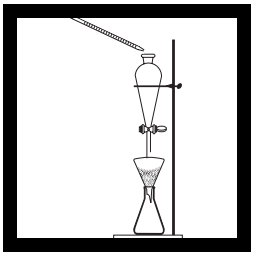
9. Return the water layer to the separatory funnel.

Note: *Use the same glass funnel for the second and third extraction (referred to in step 10).*

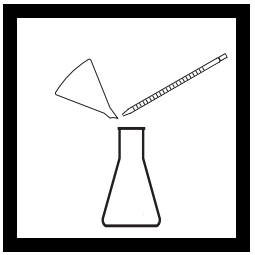
Note: *A second funnel can be used to pour the water layer into the separatory funnel to reduce spillage.*

10. Repeat *steps 4–9* two more times. After the third extraction, discard the water layer.

Note: *There may be small amounts of acetone and/or n-hexane in the water layer. See your waste disposal procedure for proper disposal instructions.*



11. Rinse the separatory funnel with three separate 5-mL aliquots of fresh n-hexane to remove any oil film left on the funnel walls. Drain each aliquot through the funnel containing the sodium sulfate into the distillation flask.

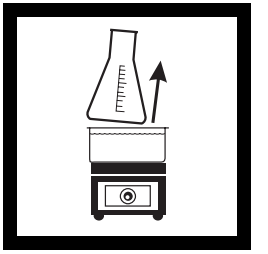


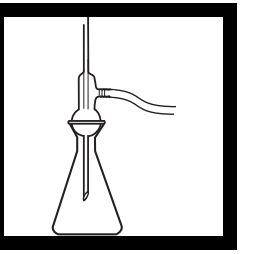
12. Rinse the tip of the glass funnel with 5 mL of n-hexane while removing it from the distillation flask. Check for sodium sulfate contamination.

Note: *Sodium sulfate contamination will appear as cubic crystals at the bottom of the distillation flask. If present, re-filter the solvent layer through filter paper without sodium sulfate. You must re-clean, dry, and weigh the boiling flask and boiling chips or have an extra flask ready in case this is necessary.*

For SGT-HEM go to step 21. For HEM go to step 14.

See Figure 1 for Distillation Assembly





13. If only the SGT-HEM is to be determined and the HEM is known, go to *step 21*. If HEM is to be determined, go to *step 14*.

Note: *If only the SGT-HEM is to be analyzed, the HEM is needed to determine the amount of silica gel needed for the SGT-HEM. For each group of samples from a discharge, determine the HEM before the SGT-HEM.*

14. Using the distillation assembly shown in *Figure 1* on page *269*, distill off the n-hexane. Distillation is complete when there are no boiling bubbles or the distillation flask appears dry.

Note: *Use a steam bath or a hot plate to maintain a water bath at the proper temperature for the distillation. Do not place the flask directly on a hot plate. This will cause low results and is dangerous because n-hexane is volatile.*

Note: *Evaporation will be faster if the long vertical arm of the connector is wrapped with insulation (paper towel, cloth, or asbestos insulating tape). The distillation should take less than 30 minutes.*

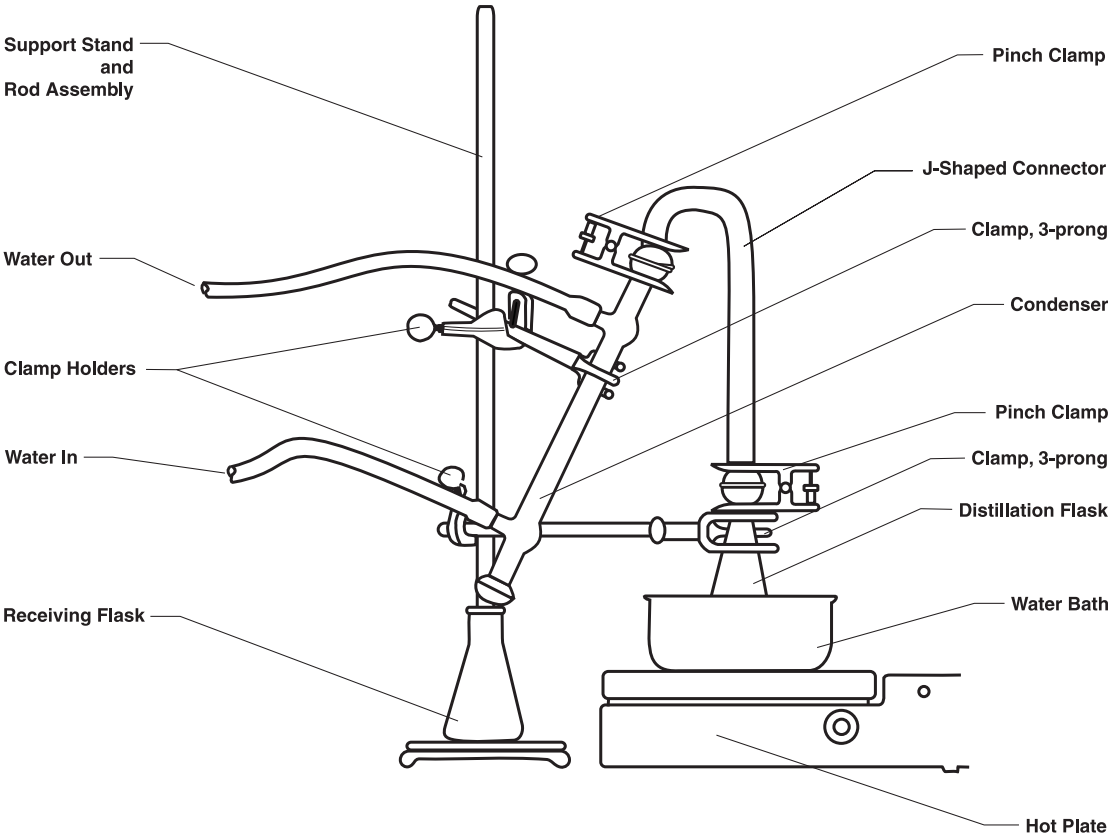
15. Disconnect the condenser/connector portion of the distillation assembly at the pinch clamp and remove the distillation flask from the heat source with an anti-lint cloth or tongs.

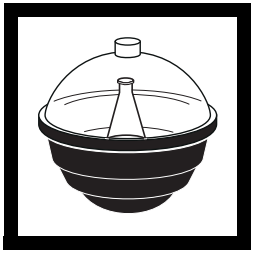
Note: *The distilled n-hexane may be re-used in future HEM extractions, but is not recommended for SGT-HEM due to the potential increased water content of the solvent.*

16. Remove the remaining solvent vapors from the distillation flask by attaching the vacuum connector/gas inlet adapter to the flask. Apply a vacuum for 1–2 minutes or until all n-hexane solvent vapors have been removed.

Note: *Crystals on the bottom of the flask indicate that sodium sulfate may have been dissolved in the extraction steps. Re-dissolve the extract in n-hexane, filter into another pre-weighed flask and repeat steps 13–15. This is not necessarily true for the "standard" extraction since stearic acid is crystalline below 69 °C. If sodium sulfate is present in the standard, big cubical crystals (not the flattened stearic acid crystals) will be visible. Also, you will calculate an unusually high yield compared to the expected value.*

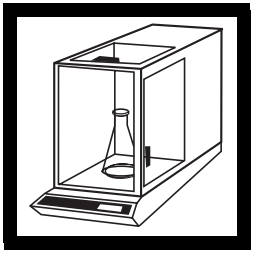
Figure 1 Distillation Assembly





17. Place the flask in a desiccator for 30 minutes (or longer if necessary) until it cools to room temperature.

Note: *If the silica gel indicator has turned red, replace the silica gel.*



18. Using an analytical balance, weigh the flask to the nearest 0.1 mg. Record this weight. Do not touch the flask after weighing; fingerprints will add weight.

Note: *Always use tongs or a lint-free wipe when handling the flask. If you touch the flask, clean the flask with lint-free wipes.*

Note: *Precise weighing is necessary for accurate results; multiple weight measurements are recommended. Re-wipe the flask before each measurement to ensure all contaminants are removed. Record each weight; use the lowest repeatable value for calculations.*

$$\frac{A - B}{\text{Sample Volume}} = \text{mg/L HEM}$$

19. Calculate the test results:

$$\frac{A - B}{\text{Sample Volume}} = \text{mg/L HEM}$$

A= Weight (mg) of flask and residue
B= Weight (mg) of flask with boiling chips.

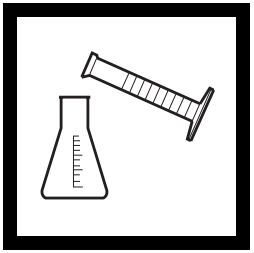
Note: *If yield is less than 15 mg/L and additional precision is needed, use a 1-liter sample.*

Example:

A = 92.4659 g
B = 92.4206 g
Sample vol. = 0.350 L

$$\frac{92.4659 - 92.4206}{0.350\ \text{L}}$$

= 0.1294 g/L (129.4 mg/L)



20. If only calculating the HEM, stop here. If continuing with SGT-HEM at Step 21, re-dissolve residue with approximately 85 mL of **fresh** n-hexane. Heat slightly to ensure all HEM materials dissolve.

Note: *For a 350 mL water sample, dilute if the HEM is >2850 mg/L (for a 1-liter sample, dilute if the HEM is more than 1000 mg/L).*

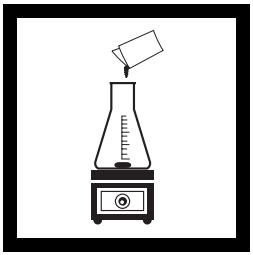
Note: *To dilute to a 1000 mg/L sample, pour the re-dissolved HEM into a 100-mL volumetric flask. Rinse the distillation flask 3-4 times with 2-3 mL of n-hexane. Fill the volumetric flask to volume with n-hexane. Mix well. Into a 100-mL beaker, volumetrically pipet the amount (V_a) determined by this equation:*

$$V_a = \frac{100000}{W_h}$$

where:

V_a = Volume of aliquot to be withdrawn (mL) to get 1000 mg of HEM.

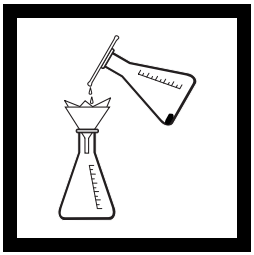
W_h = Weight of HEM (A-B in step 19 (mg)). Dilute to about 100 mL with n-hexane.



21. Put a magnetic stir bar and the correct amount of silica gel based on the equation below into the flask with the solvent/product from *step 20*.

$$\frac{3 \times \text{mg of HEM}}{100} = \text{silica gel (g } \pm 0.3)$$

22. Stir solution on a magnetic stirrer for a minimum of five minutes.



23. Pre-clean, dry and weigh a distillation flask with 3–5 boiling chips in it. Place a funnel on the distillation flask. Place a 12.5 cm filter paper in the funnel. Pre-moisten the filter paper with fresh n-hexane. Filter the solution through filter paper.

Rinse the beaker containing remaining silica gel 3 times with 5 mL aliquots of fresh n-hexane and pour the aliquots into the distillation flask.

Note: *Any spillage will cause inaccurate results. To reduce spillage, use a glass rod as a guide while pouring solution into filter.*

24. Follow *steps 14-19*. Weigh the product left in the bottom of the flask and calculate your results using the equation below:

$$\frac{A - B}{\text{Sample Volume}} = \text{mg/L SGT-HEM}$$

where:

A = Weight (mg) of flask and residue
B = Weight (mg) of flask with boiling chips.

Sampling and Storage

Collect samples in wide-mouth glass bottles or directly in the separatory funnel for immediate analysis. Collection of sample may be done directly into the separatory funnel. Measure 350 mL of water with a graduated cylinder. Pour this into the separatory funnel. Use a laboratory pen to mark the 350-mL level. Fill with sample to this mark. Do not pre-rinse the bottle or separatory funnel with the sample.

If analysis is delayed for more than a few hours, add 6 mL of 1:1 Hydrochloric Acid Solution for each liter or quart of sample. Check the pH to ensure it is 2 or less. Do not place the pH paper directly into the sample, but dip a glass rod into the sample and touch the pH paper with a drop of sample. Rinse the rod with n-hexane directly back into sample container after the pH has been determined to ensure no product has adhered to the glass rod. If necessary, add more acid to bring pH below 2. Store the sample between 04 °C (32-39 °F). Preserved samples can be stored for 28 days.

Handling Glassware

Before analysis, careful cleaning and drying of the glassware and boiling chips is necessary. Clean the chips and distillation flask by washing with hot water and detergent, rinsing with distilled water, and then rinsing with acetone or n-hexane. Place the cleaned flask and boiling chips in a drying oven at 105-115 °C for two hours. Cool to room temperature in a desiccator for at least 30 minutes. Store in the desiccator until needed.

To eliminate errors, always handle the flask with tongs or an anti-lint wipe. If the same flasks are used repeatedly, record their weights after drying in the oven without boiling chips. The drying step may be skipped if the flasks weigh the same after the acetone or n-hexane rinse as it does after drying. Boiling chips will vary in weight; their weight should be added to the flask weight.

Definition of HEM and SGT-HEM

Oil and grease is the conventional term used to define pollutants of this nature. The new term “n-hexane extractable materials” (HEM) indicates this method may be applied to materials other than oils and greases.

Likewise, the term “total petroleum hydrocarbons” (TPH) was traditionally used to classify aliphatic hydrocarbon materials. The new term “silica gel treated n-hexane extractable material” (SGT-HEM), indicates that this method may be applied to materials other than aliphatic petroleum hydrocarbons that are not adsorbed by silica gel.

Limit of Detection

This method is not applicable to measurements of materials that volatilize at temperatures below approximately 85 °C. Petroleum fuels from gasoline through #2 fuel oil may be partially lost in the solvent removal operation. Some crude oils and heavy fuel oils contain a significant percentage of materials that are not soluble in n-hexane. Recoveries of these materials may be low.

The method is capable of measuring HEM and SGT-HEM in the range of 15 to 3000 mg/L when using a 350 mL sample, and may be decreased to as low as 5 mg/L if a 1 liter sample is used. When using the 1-liter sample volume, use the amount of reagents listed for the one liter size in the EPA Monitoring and Testing Procedures and Modifications section.

Standard Preparation

1. Transfer 400 ±4 mg stearic acid and 400 ±4 mg hexadecane into a 100-mL volumetric flask.

2. Pour 75 mL of acetone into the flask. Cover with a watch glass and stir gently. Heat ***slightly*** until all material is in solution. Over-heating with the watch glass on causes pressure buildup.

Note: *Acetone (step 2) is highly flammable. Do not use near an open flame.*

3. Fill to volume with acetone. Cover. Allow to equilibrate to room temperature and continue to fill to volume until solution is at stable volume.

4. Using a volumetric pipet, transfer 5 mL of the solution from *step 3* into 350 mL of deionized reagent water. This standard solution should be 114.3 mg/L HEM or 57.1 mg/L SGT-HEM. If using a 1-liter water sample, 5 mL gives a 40 mg/L HEM and a 20 mg/L SGT-HEM. To verify the concentration, pipet 5 mL of the solution from *step 3* in a pre-weighed flask, place in hood, and allow acetone to evaporate off. Weigh the flask. Verify that the weight difference before and after solution addition is 40 ±1 mg.

Interferences

Substances extracted from samples will vary from source to source, depending upon the diversity of the site being sampled. Some samples may contain high amounts of detergents or particulates that can interfere with the extraction procedure. For these samples, it may be necessary to use a 350-mL sample size rather than one liter (which is optional). In this circumstance, the 350 mL sample size is EPA accepted for reporting. Wash all glassware in hot water with detergent, rinse with tap and distilled water and rinse with n-hexane or acetone.

If an emulsion forms between the two phases (at *step 6*) and is greater than one-third the volume of the solvent layer, filter the emulsion and solvent layer through a funnel with glass wool in it. If an emulsion still exists, other possible solutions include: stirring the solvent and emulsion layer with a stir bar, using solvent phase separation paper, centrifugation, using an ultrasonic bath with ice, addition of NaCl, or other physical methods (solid phase or other extraction techniques would fall under performance based modifications).

A milky solvent/product layer in the distillation flask indicates water in the solvent layer. Let the flask stand one hour to allow the water to settle. Re-filter the solvent layer through sodium sulfate to remove remaining water.

Extremely low yields could mean a poor extraction (*steps 5* through *8*) and a high yield could indicate a problem in the solvent drying (*step 8*). Follow procedure *steps 5–8* very carefully and run your blank before you run samples in order to identify any possible interference due to these steps. If your blank indicates a yield above 1 mg per test, you should identify the source of contamination before continuing. Likely sources are sodium sulfate contamination and improperly rinsed glassware.

EPA Monitoring and Testing Procedures and Modifications

If the Oil and Grease tests are used for compliance reporting to the USEPA, make the following changes to the procedure:

1. Use a 1-liter sample in a 2000 mL separatory funnel rather than a 350-mL sample in a 500 mL separatory funnel (*step 1*).

2. Use 6 mL (instead of 4 mL) of 1:1 hydrochloric acid to bring the pH below 2 (*step 2*), and 30 mL of n-hexane instead of 20 mL of n-hexane for the extraction (*step 4*).

Before beginning to test real samples for oil and grease, you must be able to obtain a MDL (Minimum Detection Limit) less than or equal to the EPA's reported MDL and to report an IPR (Initial Precision and Recovery). ***Before attempting the MDL and IPR, it is highly recommended to run laboratory reagent water blanks to eliminate all interference.***

MDL: The USEPA-documented MDL is extremely difficult to reproduce if the slightest sodium sulfate contamination occurs. EPA Method 1664 requires the MDL to be ≤1.4 mg/L for HEM and ≤1.6 mg/L for SGT-HEM. This is calculated by determining the standard deviation of seven standard samples for HEM or seven standards for SGT-HEM and multiplying their respective standard deviations by 3.143 (Student's *t* test).

The recommended standard concentration for determining the MDL is about 5 mg/L. To prepare the standard for HEM follow *steps 1-3* in *Section* on page *273*, but change *step 1* to transfer 100 ±4 mg stearic acid and 100 mg ±4 mg hexadecane to a 250-mL volumetric flask. To prepare the SGT-HEM standard, transfer 200 ±4 mg of decahexane only into a 250-mL volumetric flask. Transfer 5 mL of either standard into one liter of reagent water. Analysis of the standard should give 5 mg/L for either HEM or SGT-HEM.

IPR: Follow the procedure for HEM and SGT-HEM, using deionized water (void of any oil and grease) as the blank. Perform the procedure four separate times using 5 mL of the standard (40 mg/L 1:1 stearic acid/hexadecane (prepared in *Section* on page *273*) diluted into one liter of deionized water. Report the average percent recovery (X) and the standard deviation of the

percent recovery (s) for both HEM and SGT-HEM. X and s should fall within these parameters:

a. **HEM:** Precision(s) ≤10%; Recovery(X) 83%–101%.

b. **SGT-HEM:** Precision(s) ≤13%; Recovery(X) 83%–116%.

If not within these ranges, correct the problem and repeat IPR.

Once the MDL and IPR have been established, keep records for EPA verification or for future reference.

Oil and Grease Reporting to EPA

To report the HEM and/or SGT-HEM, include the following data for each set of up to ten samples.

1. **BLANK:** Must be less than 5.0 mg/L for HEM and SGT-HEM.

2. **OPR** (Ongoing Precision and Recovery): Determine the amount of analyte in a one-liter sample containing 5 mL of the standard (40 mg/L 1:1 stearic acid/hexadecane). Continue on if the HEM recovery is 70%–114% and the SGT-HEM recovery is 66%–114%. If recovery is lower, an interference may be present or analysis technique may be faulty. Identify the cause and repeat OPR until within range**.**

3. **MS and MSD** (matrix spike and matrix spike duplicate): Determine the background concentration (B) of your sample by running HEM and SGT-HEM on the discharge water. Spike two one-liter discharge water samples with 5 mL of standard and measure concentration of analyte after spiking (this is value A).

Note: *For compliance monitoring, it may be necessary to use a standard that either matches the regulatory concentration limit or is 1 to 5 times higher than the concentration of the sample (B), whichever is greater. The concentration of the spike (T) needs to be divided by 2 for SGT-HEM if using the standard (40 mg/L 1:1 stearic acid/hexadecane).*

Determine the Percent Recovery (P) as follows:

$$P_{HEM\ (40mg/L)} = \frac{100 \times (A - B)}{T}$$

$$P_{SGT\text{-}HEM} = \frac{100 \times (A - B)}{T/2}$$

If HEM Recovery is 79–114% and SGT-HEM Recovery is 66%–114%, then calculate the Relative Percent Difference (RPD). If recovery is lower, an interference may be present. Identify and correct the interference, then repeat MS and MSD.

$$RPD = \frac{|Conc_{MS} - Conc_{MSD}|}{Conc_{MS} + Conc_{MSD}} \times 200$$

If RPD for HEM≤18 and RPD for SGT-HEM≤24, then continue with *step 4*. If recovery is lower, an interference may be present. Identify and correct the interference, then repeat MS and MSD.

After every five MS/MSD tests, compute the average percent recovery (P_a) and standard deviation of the percent recovery (s_p). Record these numbers as $P_a \pm 2s_{p.}$

4. **Calibrate** your analytical balance at 2 mg and 1000 mg using class "S" weights to ensure calibration within ±10%.

5. **Analyze up to 10 samples** from the source used in the MS and MSD before starting back at step 1. Every 10 samples you must determine a new blank, OPR, RPD, MS and MSD.

Summary: Each laboratory must first verify the MDL and IPR and ensure they are within proper parameters before reporting Oil and Grease test results to the EPA. Once this is established for a laboratory, it does not need to be done again.

For every 10 samples per discharge source, one must calibrate the balance, report one blank, one OPR, one MS, and one MSD. Logs must be kept on percent recovery and relative percent differences for MS/MSD tests. For every five MS/MSD tests, calculate and record the average percent recovery and standard deviation.

Summary of Method

Oil and Grease & Total Petroleum Hydrocarbons (TPH) include any material that may be recovered as a substance that is soluble in the n-hexane extractant. These include substances such as relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases, and related materials. When measuring oil and grease (HEM) gravimetrically, the substances are extracted from the sample with n-hexane, then the n-hexane is evaporated. The residue left is weighed to determine the concentration of oil and grease materials in mg/L.

When measuring Total Petroleum Hydrocarbons (SGT-HEM) gravimetrically, the substances are extracted from the sample with n-hexane, then mixed with silica gel to absorb non-TPH components. Then the n-hexane is evaporated. Like the HEM, the residue left is weighed to determine the concentration of total petroleum hydrocarbons. Careful technique is required to obtain accurate and precise results.

REQUIRED REAGENTS

Description	Quantity required per test	Unit	Cat No.
Acetone, ACS	varies	500 mL	14429-49
Hexadecane, 99%	400 mg	100 mL	26648-42
Hydrochloric Acid Standard Solution, 1:1 (6N)	4 mL	500 mL	884-49
Hexane, ACS grade	<200 mL	500 mL	14478-49
pH Paper, 0–14 pH units	varies	100/pkg	26013-00
Silica Gel with indicator (for desiccator)	varies	454 g	14269-01
Silica Gel, 100–200 mesh	1-30 g	500 g	26650-34
Sodium sulfate, anhydrous	10 g	113 g	7099-14
Stearic Acid	400 mg	500 gm	26649-34

REQUIRED APPARATUS

Adapter, vacuum connector/gas inlet, 28/15	1	each	14339-00
Aspirator, vacuum	1	each	2131-00
Balance, analytical (0.001–210 g, reads to 0.1 mg)	1	each	26103-00
Boiling Chips	3–10	500 g	20557-34
Clamp, pinch type, No. 28	2	each	14338-00
Clamp, 3-prong	2	each	422-00
Clamp, holder	2	each	326-00
Condenser, reflux, w/ground glass joints, 28/15	1	each	14337-00
Cylinder, graduated, 500 mL	1	each	20885-49
Cylinder, graduated, 50 mL	1	each	508-41
Desiccator	1	each	20888-00
Desiccator Plate	1	each	14284-00
Filter Funnel, 65 mm, short stem	1	each	26647-00
Filter Paper, 12.5 cm, folded	1	100/pkg	692-57
Flask, Erlenmeyer, 125 mL	1	each	505-43
Flask, Erlenmeyer, 125 mL, w/ground glass joint 28/15	2	each	14340-00
Funnel, separatory, 500 mL	1	each	520-49
Hot Plate, 120 V, 50 Hz	1	each	23441-00
Oven, drying, 120 V, 50 Hz	1	each	14289-00
Pen, Laboratory	1	each	20920-00
pH Paper, 0-14 pH units	varies	100/pkg	26013-00
Pipette filler, safety bulb	1	each	14651-00
Pipette, serological, 5 mL	1	each	532-37
Rod, glass	1	3/pkg	1770-01
Separatory funnel, 2 liters	1	each	520-54
Stirrer, magnetic 120 V	1	each	23436-00
Stir Bar, 22.2 x 7.9 mm	1	each	20953-50
Support stand	1	each	563-00

REQUIRED APPARATUS (continued)

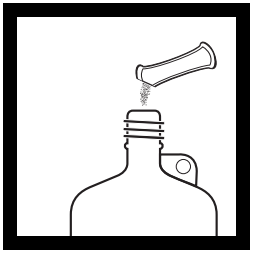
Description	Quantity required per test	Unit	Cat No.
Tongs, crucible, 9-inch	1	each	569-00
Tube, connecting, J-shaped, w/ground glass joint, 28/15	1	each	18143-00
Tubing, black rubber, 7.9 x 2.4 mm	1	3.6 m	560-19
Steam bath, 8-inch, 5-ring	1	each	23479-00

OPTIONAL APPARATUS

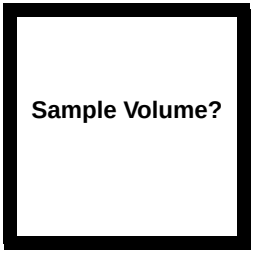
Beaker, 400 mL (for SGT-HEM dilution)	each	500-48
Bottle, Storage, glass, amber, 473 mL	6/pkg	7144-21
Bottle, Storage, glass, amber, 1000 mL	6/pkg	7144-63
Cap, polypropylene (for 473 mL bottle)	6/pkg	21587-06
Cap, polypropylene (for 1000 mL bottle)	6/pkg	23710-06
Hot Plate, 240 V, 60 Hz	each	23441-02
Oven, drying, 240 V, 60 Hz	each	14289-02
Ring Stand, 5-inch ID	each	26831-00
Stirrer, magnetic, 240 V	each	23436-02
Thermometer, -10 to 110 °C	each	1877-01
Vacuum pump, hand operated (Nalgene)	each	14283-00
Volumetric flask, 100 mL	each	14574-42
Volumetric flask, 500 mL	each	14574-49
Volumetric flask, 1000 mL (if using 1 L sample)	each	14574-53
Volumetric pipet, 5 mL	each	14515-37

OXYGEN DEMAND, BIOCHEMICAL

Dilution Method* (USEPA Accepted)

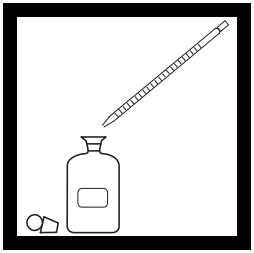


1. Prepare sample dilution water using a BOD Nutrient Buffer Pillow; see *Dilution Water Preparation* following this procedure.



2. Determine the range of sample volumes required for your sample; see *Choosing Sample Size* following this procedure.

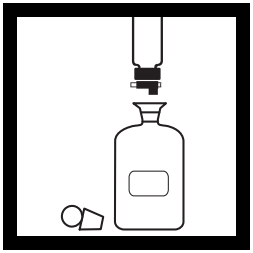
Note: *If the minimum sample volume is 3 mL or more, determine the dissolved oxygen in the undiluted sample. This can be omitted when analyzing sewage and settled effluents known to have a dissolved oxygen content near 0 mg/L.*



3. With a serological pipet, measure a graduated series of at least four, but preferably five or six, portions of well-mixed sample and transfer to separate 300-mL glass-stoppered BOD bottles. Stir the sample with the pipet before pipetting each portion.

Note: *See Interferences following this procedure when analyzing chlorinated or industrial effluents.*

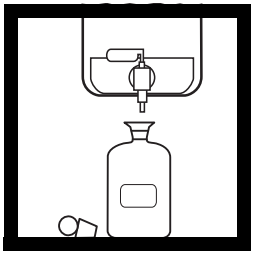
Note: *Do not add sample to one BOD bottle. This will be the dilution water blank. For additional proof of accuracy, see Accuracy Check following these steps*.



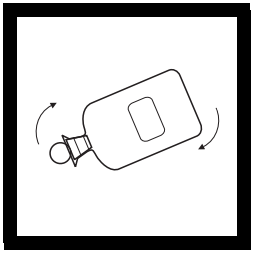
4. Add two shots of Nitrification Inhibitor (approx. 0.16 g) to each bottle, if desired.

Note: *Use of Nitrification Inhibitor will inhibit the oxidation of nitrogen compounds if only carbonaceous oxygen demand is desired. It is especially recommended for samples with low BODs.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater* and from Klein, R.L., Gibbs, C., *Journal Water Pollution Control Federation*, 51 (9), 2257 (1979).



5. Fill each bottle to just below the lip with seeded or unseeded dilution water. When adding the water, allow it to flow slowly down the sides of the bottle to prevent formation of bubbles.

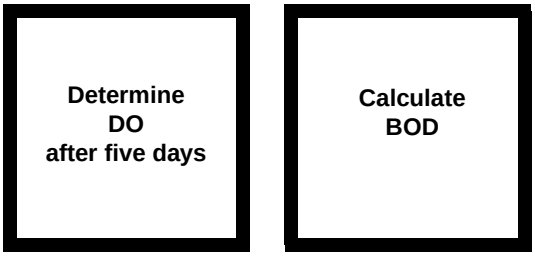


6. Stopper the bottle, being careful not to trap any air bubbles. Press on the stopper of the bottle with your finger; then invert the bottle several times to mix.

Note: *Determine initial DO's if following the Standard Methods' procedure. This is not necessary if using the graphical method.*

7. Add enough dilution water to the lip of the BOD bottle to make a water seal.

8. Place a plastic overcap over the lip of each bottle and place bottles in an incubator at 20 ± 1° C. Incubate in the dark for five days.



9. When the five-day incubation period is complete, determine the dissolved oxygen content (mg/L DO remaining) in each bottle as described in the *Dissolved Oxygen, Procedure* in this manual, or potentiometrically by using a dissolved oxygen probe.

Note: *This procedure has been EPA approved. But, the graphical method outlined in step 10 has not. See Calculating Results: Standard Methods (following these steps), for the EPA Approved calculation.*

10. Determine the BOD using the graphical method as follows; see Calculating Results: Grapical Method for more information.

a. Plot the mg/L DO remaining in each diluted sample versus the mL sample taken; then draw the best straight line through the plotted points.

Note: *An erroneous point is visually evident and can be disregarded. However, at least three points should be on the line or very close to it. For unseeded dilution water, the line should cross the "mg/L oxygen remaining" scale near or below the oxygen saturation value for the altitude of the laboratory (see* Dilution Water Preparation*).*

b. To calculate the BOD, use the following equation which is mathematically equivalent to the BOD equation in Standard Methods.

mg/L BOD =
(A x 300) - B + C

Where:

A = the slope. The slope of the line is equal to the mg/L DO consumed per mL of sample taken. Take any point on the line and subtract the mg/L DO remaining at that point from the mg/L DO where the line crosses the DO scale (Y intercept, mg/L DO remaining). Divide the difference by the mL of sample at the point chosen.

300 = the volume of the BOD bottle

B = the Y intercept. This is the DO value where the line crosses the "DO remaining" scale. (This should be very close to the actual dilution water blank value.)

C = the sample DO. This is the DO of the undiluted sample.

Another way to write this equation is:

mg/L BOD =

(Slope x 300) -

Y intercept + Sample DO

Note: *If the best straight line is obtained by linear regression through use of a calculator, the sign (-) of the slope must be changed (+) before multiplying by 300.*

Dilution Water Preparation

Note: *The DO uptake in five days at 20 °C should not exceed 0.2 mg/L.*

The BOD test requires very high quality water be used for diluting samples. Water must be free of all toxic substances, such as small amounts of chlorine, copper and mercury, as well as free of organic matter. If organic matter is present in dilution water, it will create an oxygen demand.

The most practical way to produce water of low organic content on a consistent basis is by distillation from alkaline permanganate (Sodium Hydroxide Pellets and Potassium Permanganate). Commercial stills which automatically produce high quality distilled water are available.

Direct use of deionized water from ion exchange columns is not recommended because of the erratic release of organic materials from the cartridges, especially new ones. These organic materials will not be detected with conductivity measurements but may show up in the final results as an oxygen demand. Bacterial growth also may be present on the column.

Distilled water, as it is produced from a still, is usually and not saturated with oxygen. The temperature of the BOD dilution water must be 20 °C at the time of use and near or at saturation with oxygen. It is recommended that distilled water be stored in a BOD incubator until it reaches 20 °C and dilution water be prepared immediately before use. The distilled water can be placed in one-gallon jugs by filling each of them with three liters or by filling two-gallon jugs with six liters. The jugs should be capped and placed in the incubator for storage. After 24 hours or more, the temperature will be 20 °C and the water will be saturated or nearly saturated with oxygen furnished by the air above the water in the jugs.

Note: *The bubbling apparatus should be cleaned before and after use.*

If five-gallon containers are used, the distilled water should be saturated with oxygen by bubbling in filtered air from a hose connected to an aquarium pump or air compressor.

It is not necessary to use seeded dilution water when analyzing sewage, sewage plant effluent (unless it has been chlorinated) or river water. However, there are certain samples such as industrial or trade wastes or chlorinated sewage which do not contain sufficient bacteria to oxidize the organic matter that is present.

To test such samples, some bacterial seed must be added to the samples. This is done by adding a small, measured volume of water known to contain a good bacterial population to the dilution water.

Raw sewage is recommended as a source of seed. This material should be stored at 20°C for 24 to 36 hours before use. When using domestic sewage as seed, it should be allowed to stand undisturbed until most solids settle. Pipet from the upper portion of the bottle of seed material. It has been found that the addition of 3.0 mL of raw domestic sewage seed to each liter of dilution water is ample. Seed that has a BOD of 200 mg/L (a typical range for domestic sewage) when added at the rate of 3 mL per liter of dilution water will deplete 0.6 mg/L DO. An alternative to raw sewage as a source of seed is USEPA-approved Polyseed® BOD Seed Inoculum (Cat. No. 24712-00), a dehydrated seed inoculum.

Using BOD Nutrient Buffer Pillows

To prepare dilution water, select the BOD Nutrient Buffer Pillow for the amount of dilution water you wish to prepare; see *Table 1*. Shake the pillow, cut it open, and add the contents to a jug containing the proper amount of 20 °C distilled water. Choose a container which will be only partially filled by the solution. Cap the jug and shake vigorously for one minute to dissolve the slurry and to saturate the water with oxygen.

Table 1

Description		Cat. No.
BOD Nutrient Buffer Pillows:		
	for preparing 300 mL of dilution water	14160-66
	for preparing 3 liters of dilution water	14861-66
	for preparing 6 liters of dilution water	14862-66
	for preparing 19 liters of dilution water	14863-98

Following Conventional Method

To prepare dilution water by the conventional method, pipet 1 mL of each of the following solutions per liter of distilled water at 20 °C: Calcium Chloride Solution, Ferric Chloride Solution, Magnesium Sulfate Solution, and Phosphate Buffer Solution. Cap the bottle and shake vigorously for one minute.
The Phosphate Buffer Solution should be refrigerated to decrease

the rate of biological growth. Use care with all solutions to avoid contamination.

Choosing Sample Size

The range of sample volumes to be diluted depends on two factors: type of sample and the laboratory's elevation.

If the sample contains high levels of organic material, such as raw sewage, its BOD will be high and small portions must be diluted in the test. If a sample has a low BOD, such as polluted river water, larger portions will be necessary; see *Table 2*.

The laboratory's elevation influences the amount of oxygen that can be dissolved in the dilution water. At sea level and normal barometric pressure, water can be saturated with up to 9.2 mg/L DO at 20 °C.

At higher elevations, the amount of oxygen that can dissolve in water decreases, so less oxygen is available to microorganisms. Refer to *Table 4*. Smaller portions of sample must be taken so there will be dissolved oxygen remaining in the BOD bottle after five days of incubation. For most accurate results, sample sizes should be chosen so that at least 2.0 mg/L of dissolved oxygen are consumed during the incubation period, but 1.0 mg/L DO is left in the BOD bottle. (*Table 2* has done this for you).

Follow these steps to determine the range of sample volumes to use:

a. Estimate the BOD of your particular sample type (see *Table 2*). Sewage has approximately 300 mg/L BOD; oxidized effluents have about 50 mg/L or less.

b. Determine the minimum sample volume that can be used for the estimated BOD of your sample from *Table 2*. For example, if a sewage sample is estimated to contain 300 mg/L BOD, the smallest allowable sample volume is 2 mL. For sewage effluent with an estimated BOD of 40 mg/L, the volume is 15 mL.

c. Determine the laboratory's altitude.

Table 2 Determining Minimum Sample Volume

Sample Type	Estimated BOD mg/L	mL of Sample*
Strong Trade Waste	600	1
Raw and Settled Sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10
Oxidized Effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted River Waters	6	100
	4	200
	2	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

d. Determine the maximum sample volume for the altitude of your laboratory from *Table 3*. At 1,000 feet an estimated BOD of 300 mg/L, the largest sample portion should be 8 mL. For a BOD of 40 mg/L the maximum volume is 60 mL.

e. Choose three other sample volumes between the minimum and maximum volumes so the total number of portions is five or greater. In the two cases given in *step d* above, a series of 2, 4, 5, 6 and 8 mL portions is suggested for an estimated BOD of 300 mg/L and a series of 15, 25, 35, 45 and 60 mL portions for a BOD of 40 mg/L.

Table 3 Determining Maximum Sample Volume

Estimated BOD at			mL of Sample*
sea level	1000 ft. 300 m	5000 ft. 1500 m	
2460	2380	2032	1
1230	1189	1016	2
820	793	677	3
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

Table 4 Oxygen Saturation Values at Various Altitudes

Oxygen Saturation Value	Sea Level	1000 ft. 300 m	2000 ft. 600 m	3000 ft. 900 m	4000 ft. 1200m	5000 ft. 1500 m	6000 ft. 1800 m
(at 20 °C)	9.2 mg/L	8.9 mg/L	8.6 mg/L	8.2 mg/L	7.9 mg/L	7.6 mg/L	7.4 mg/L

Interferences

Many chlorinated and industrial effluents require special handling to ensure reliable BOD results. Usually, careful experimentation with the particular sample will indicate what modifications should be made to the test procedure.

Toxins in the sample will adversely affect any microorganisms present and result in lower BODs.

1. To eliminate small amounts of residual chlorine*, allow the sample to stand for one to two hours at room temperature. For larger quantities, determine the amount of sodium thiosulfate to add to the sample as follows:

a. Measure 100 mL of sample into a 250-mL Erlenmeyer flask. Using a 10-mL serological pipet and a pipet filler, add 10 mL of 0.020 N Sulfuric Acid Standard Solution and 10 mL of Potassium Iodide Solution, 100 g/L, to the flask.

b. Add three full droppers of Starch Indicator Solution and swirl to mix.

c. Fill a 25-mL buret with 0.025 N Sodium Thiosulfate Standard Solution and titrate the sample from dark blue to colorless.

d. Calculate the amount of 0.025 N Sodium Thiosulfate Standard Solution to add to the sample:

$$\text{mL 0.025N sodium thiosulfate required} = \frac{\text{mL titrant used x volume of remaining sample}}{100}$$

e. Add the required amount of 0.025 N Sodium Thiosulfate Standard Solution to the sample. Mix well. Wait 10 to 20 minutes before running the BOD test.

2. To eliminate the effect of phenols, heavy metals or cyanide, dilute the sample with high quality distilled water. Alternately, the seed used in the dilution water may be acclimatized to tolerate such materials. Acclimatize seed as follows:

a. Fill a one-gallon stainless steel or plastic container with domestic sewage and aerate for 24 hours. Allow the heavier material to settle.

b. After settling for one hour, siphon off three quarts of material and discard.

* Measure chlorine with Hach Water Quality Chlorine Test Strips (Cat. No. 27450-50).

c. Fill the container with a mixture of 90% sewage and 10% wastes containing the toxic material.

d. Aerate for 24 hours. Repeat *steps b* and *c* with increasing amounts of waste until the container holds 100% toxic waste material.

3. Optimum pH for BOD test is between 6.5 and 7.5. Adjust samples to pH 7.2 with Phosphate Buffer Solution or 1 N (or more dilute) Sulfuric Acid or Sodium Hydroxide Standard Solution if the pH is not in this range.

4. Cold samples may be supersaturated with oxygen and will have low BOD results. Fill a one-quart bottle about halfway with cold sample and shake vigorously for two minutes. Allow sample temperature to reach 20 °C before testing.

Calculating Results

Graphical Method

The mg/L DO remaining was determined for a series of four dilutions of domestic sewage after five days of incubation. Results were as follows:

mL of sample taken	mg/L DO remaining
2.0	7.50
3.0	6.75
6.0	4.50
9.0	2.25

The DO values were plotted versus the mL of sample taken and a straight line drawn as in *Figure 1*. If a set of BOD dilutions is run correctly with a homogeneous sample, a graph of the mg/L DO remaining versus the sample volume should result in a straight line. The value where the line intersects the y axis is equal to the DO content of the dilution water after incubation, although this is not actually measured. In this case, it was equal to 9.0 mg/L and the DO of the domestic sewage sample was assumed to be zero. If another type of sample is used, the DO of an undiluted sample should be measured either by the Winkler titration or potentiometrically.

The American Public Health Association formula for calculating BOD also can be written as follows (not approved for reporting purposes):

$$\frac{\text{mg/L DO remaining (smaller sample)} - \text{mg/L DO remaining (larger sample)}}{\text{mL (larger sample)} - \text{mL (smaller sample)}} \times 300 - DO_D + S = \text{BOD}$$

Using this information in the previous example:

mg/L DO remaining with smaller sample volume = 7.50

mg/L DO remaining with larger sample volume = 2.25

mL of larger sample volume = 9.0

mL of smaller sample volume = 2.0

300 = volume (mL) of BOD bottle

DO_D = mg/L DO of dilution water = 9.0

S = mg/L DO of sample = assumed in this case to be zero

Therefore:

$$\frac{(7.50 - 2.25)}{(9.0 - 2.0)} \times 300 - 9 + 0 = \text{mg/L BOD}$$

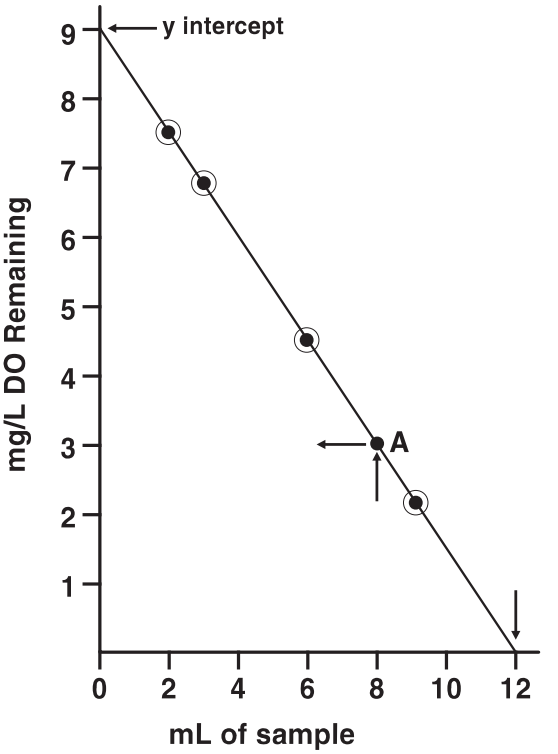
$$\frac{5.25}{7} \times 300 - 9 =$$

$$0.75 \times 300 - 9 =$$

$$225 - 9 =$$

$$216 = \text{mg/L BOD}$$

Figure 1



Using the equation in step 10:

$$(\text{slope} \times 300) - \text{Y intercept} + \text{sample DO} = \text{mg/L BOD}$$

Where:

slope = we arbitrarily select point A in Figure 1. At this point the mg/L DO remaining is equal to 3.0 mg/L. The mL of sample at this point is 8 mL. The difference between the Y intercept of 9.0 mg/L and 3.0 mg/L equals 6 mg/L. 6 mg/L divided by 8 mL = 0.75 mg/L per mL.

Y intercept = 9.0 mg/L

sample DO = 0; because the sample is domestic sewage, this was assumed to be 0.

Therefore:

$$(0.75 \times 300) - 9.0 + 0 = \text{mg/L BOD}$$

$$225 - 9 = 216 \text{ mg/L BOD}$$

Standard Methods*

When dilution water is not seeded:

$$BOD_5, mg/L = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$BOD_5, mg/L = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

Where:

D_1 = DO of diluted sample immediately after preparation, mg/L

D_2 = DO of diluted sample after 5 d incubation at 20 °C, mg/L

P = decimal volumetric fraction of sample used

B_1 = DO of seed control before incubation, mg/L

B_2 = DO of seed control after incubation, mg/L

f = ratio of seed in diluted sample to seed in seed control = (% seed in diluted sample)/(% seed in seed control)

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ if nitrification is inhibited. If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average results in the acceptable range.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

Accuracy Check

A standard mixture of glucose and glutamic acid is available to check the accuracy of BOD results. Follow the directions below:

a. Snap the neck off a BOD Voluette® Ampule Standard Solution for BOD, dilution method.

b. Using Class A volumetric pipets and a pipet filler, pipet 1.00, 2.00, 3.00, and 4.00 mL of standard into four BOD bottles.

c. Fill bottles with seeded dilution water and incubate at 20°C for five days.

d. Determine the DO remaining in each bottle; then plot the mg/L remaining DO versus the volume of standard used.

e. Draw the best straight line through the plotted points.

f. Determine the BOD of the standard according to *step 10* of the procedure.

g. Divide the value by two.

Note: *Since the BOD standard contains 300 mg/L each of glucose and glutamic acid, the BOD value determined from the graph must be divided by 2 to correspond with values reported in Standard Methods. Your result should be within the standard deviation listed.*

Note: *On the basis of a mixed primary standard containing 150 mg/L each of glucose and glutamic acid, Standard Methods determines that the average five-day BOD would be 198± 30.5 mg/L*.*

* Data taken from *Standard Methods for the Examination of Water and Wastewater, 19th ed., p.5-6* (**1995**).

Summary of Methods

Biochemical Oxygen Demand (BOD) is an empirical measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters. The BOD test is of limited value in measuring the actual oxygen demand because temperature change, biological population, water movement, sunlight, oxygen concentration and other environmental factors cannot be reproduced accurately in the laboratory. The BOD test is of greatest value after patterns of oxygen uptake for a specific effluent and receiving water have been established.

The BOD is performed by incubating a sealed wastewater sample (or a prepared dilution) for the standard five-day period and then determining the change in dissolved oxygen content. The BOD value can then be calculated from the results of the dissolved oxygen tests.

REQUIRED REAGENTS AND APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
BOD Nutrient Buffer Pillows, preparing 3 liters of dilution water	1	50/pkg	14861-66
Bottle, glass-stoppered, 300 mL	6	each	621-00
Bottle, wash, 500 mL	1	each	620-11
Clippers, large	1	each	968-00
BOD Bottle Cap	6	6/pkg	2419-06
Pipet Filler	1	each	12189-00
Select one or more based on sample volume:			
Pipet, serological, 1 mL	varies	each	532-35
Pipet, serological, 5 mL	varies	each	532-37
Pipet, serological, 10 mL	varies	each	532-38

OPTIONAL REAGENTS

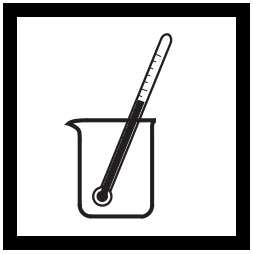
Description	Unit	Cat. No.
BOD Standard Solution, Voluette™ ampule, 300 mg/L, 10 mL, dilution method	16/pkg	14865-10
BOD Nutrient Buffer Pillows		
for preparing 300 mL of dilution water	50/pkg	14160-66
for preparing 6 liters of dilution water	50/pkg	14862-66
for preparing 19 liters of dilution water	25/pkg	14863-98
Buffer Solution, APHA, for BOD, pH 7.2, phosphate type	1 L	431-53
Calcium Chloride Solution, APHA, for BOD	1 L	428-53
Ferric Chloride Solution, APHA, for BOD	1 L	429-53
Magnesium Sulfate Solution, APHA, for BOD	1 L	430-53
Nitrification Inhibitor	35 g	2533-35
Polyseed® BOD Inoculum	50/pkg	24712-00
Potassium Iodide Solution, 100 g/L	500 mL	12289-49
Potassium Permanganate	454 g	168-01
Sodium Hydroxide, pellets	500 g	187-34
Sodium Hydroxide Standard Solution, 1.000 N	100 mL MDB	1045-32
Sodium Thiosulfate Standard Solution, 0.025 N	1 L	352-53
Starch Indicator Solution	100 mL MDB	349-32
Sulfuric Acid Standard Solution, 0.020 N	1 L	203-53
Sulfuric Acid Standard Solution, 1.000 N	1 L	1270-53

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Bottle, 4 L, with spigot	each	14868-17
Bottle, 10 L, with spigot	each	14868-58
Buret, Teflon stopcock, 25 mL	each	14681-40
Clamp, buret double	each	328-00
Cylinder, graduated, 100 mL	each	508-42
Dispenser Cap, for Nitrification Inhibitor (35 bottle only)	each	459-01
Flask, Erlenmeyer, 250 mL	each	505-46
Meter, Dissolved Oxygen, *sens**ion**™6*, portable, w/ probe	each	51850-10
Pipet, serological, 25 mL	each	2066-40
Sampler, sewage	each	427-00
Still, laboratory, 2–3 liter/hour, 110 V	each	26318-00
Support Base and Rod	each	329-00
Thermometer, -10 to 110 °C	each	1877-01

OXYGEN DEMAND, BIOCHEMICAL

Respirometric Method (using the BODTrak apparatus)



1. Heat or cool the sample to within 2 °C of its incubation temperature (typically 20 °C (68 °F).

2. Using a clean graduated cylinder, pour the correct sample volume into a BODTrak sample bottle (see *Table 1*). See the *Sample Dilutions* section for more information on BOD range selection.

3. Place a 3.8-cm (1½-in.) magnetic stir bar in each sample bottle.

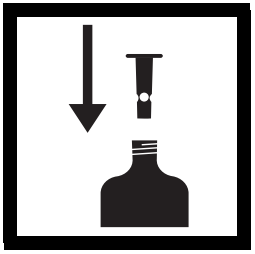
4. Add the contents of one BOD Nutrient Buffer Pillow to each bottle for optimum bacteria growth.

Note: *Step 4 is optional. If simulation of original sample characteristics is required, do not add the BOD Nutrient Buffer.*

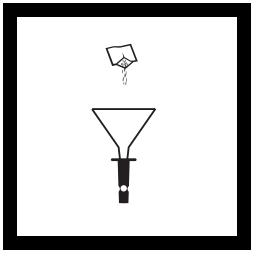
Table 1 Selection of Sample Volume

BOD Range (mg/L)	Required Volume (mL)
0-35	420
0-70	355
0-350	160
0-700	95

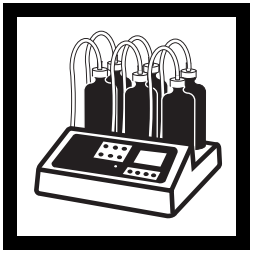
5. Apply stopcock grease to the seal lip of each bottle and to the top of each seal cup.



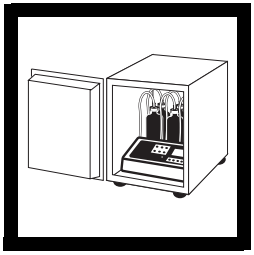
6. Place a seal cup in the neck of each bottle.



7. Using the funnel, add the contents of one Lithium Hydroxide Powder Pillow to each seal cup. Do not allow lithium hydroxide particles to fall into the sample. If this occurs, discard the sample and prepare a fresh one.

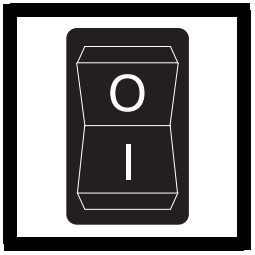


8. Place the bottles on the base of the BODTrak. Connect the appropriate tube to the sample bottle and firmly tighten the cap. Each tube is tagged with the channel number, and the channel number setup will be reflected on the control panel.



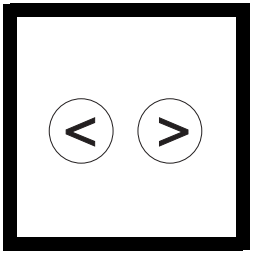
9. Place the instrument in the incubator.

Note: *The American Public Health Association (APHA) recommends a solution incubation temperature of 20 ±1 °C (68 ±1 °F) for the BOD test. Adjust your incubator to the appropriate temperature setting for each sample volume used in this test. This temperature value varies with incubator circulation.*

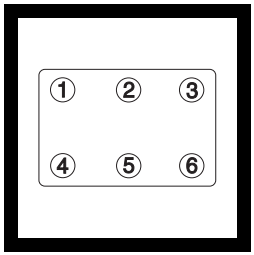


10. Start the instrument (connect the electrical plug and turn the instrument on).

11. Make sure all stir bars are rotating. If a stir bar slides to the side of the bottle, lift the bottle off the unit and gently replace. Do not start the channel until the stir bar is rotating properly.



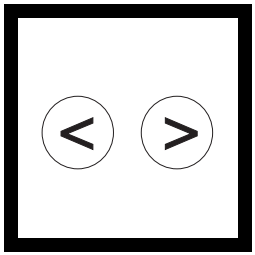
12. To select a test duration, simultaneously press and hold the left and the right arrow keys until the time menu appears. Press the **CHANNEL 6** key to activate the test length parameter. Use the arrow keys to choose a 5-, 7-, or 10-day test (test length is shown on the last line of the screen). Press **OFF** to save selections and exit the menu.



13. To start a test, press the channel number corresponding to the sample bottle.

Note: *Each channel (1–6) must be started individually.*

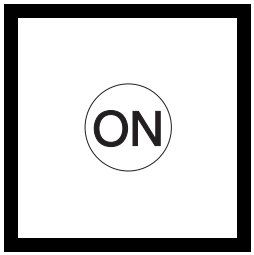
14. Press the **ON** key. A menu for selecting the BOD range will be displayed.



15. For 0–350 mg/L range, press the right arrow key. For 0–700 mg/L press the right arrow key a second time.

For 0–35 mg/L range, press the left key.

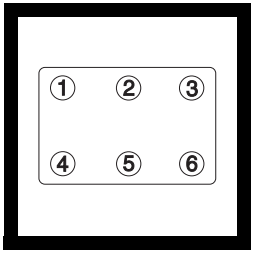
For 0–70 mg/L press the left arrow key a second time.



16. Press and hold the **ON** key to start a test. A graph will be displayed. To cancel a test, press **OFF.**

Note: *Repeat steps 13 to 16 for each channel used.*

Note: *The BODTrak automatically stops each channel after the selected time has passed. A channel can be manually stopped by depressing the* ***OFF*** *key for several seconds. The display status will change from RUN to END.*



17. Read the BOD results directly from the BODTrak display by pressing the key corresponding to each sample channel.

Note: *See Downloading Test Results in the Instrument Manual for information on transferring data to a computer or printer.*

18. Use a brush and hot soapy water to clean all bottles, stir bars, and seal cups. Rinse thoroughly with distilled water.

Sampling and Storage

For best results, analyze samples immediately after collection. If this is not possible, preserve samples at low temperature (4 °C) for no longer than 24 hours.

Sample Dilutions

If the sample's BOD is unknown, you can generally assume that effluent is normally in the 0-70 mg/L range while influent is usually in the 0-700 mg/L range.

If a sample does not contain sufficient nutrients for optimum bacteria growth, add the contents of one BOD nutrient Buffer Pillow to each bottle. Do not add the BOD Nutrient Buffer Pillow if close simulation of original sample characteristics is required.

When Oxygen Demand Exceeds 700 mg/L

When the O_2 demand of a sample exceeds 700 mg/L, dilute the sample with high-quality dilution water. Make the dilution water with distilled water that does not contain organic matter or traces of toxic substances such as chlorine, copper, and mercury.

Demineralizers can release undetected organic matter that will create an objectionable oxygen demand. The most practical way to consistently produce water of low organic content is by distillation from alkaline permanganate. (For example, add 2 g $KMnO_4$ and 4 g NaOH for every liter of water.)

After distillation, place 3 L of distilled water in a jug and bring the water temperature to 20 °C. Add the contents of one BOD Nutrient Buffer Pillow for 3 L to ensure sufficient nutrient concentration for diluted samples. Cap the jug and shake it vigorously for one minute to saturate the water with oxygen.

Do not store the solution.

Preparing Several Identical Samples
Perform a single dilution for all samples when several identical samples are needed. After the dilution, multiply the reading by the dilution factor.

Example:
Prepare a 1:5 dilution by multiplying the original sample volume by 5 and adding dilution water until the new volume is obtained. If the sample volume is 200 mL:

$5 \times 200 = 1000$ mL

Dilute the 200-mL sample to 1000 mL using the dilution water. Multiply the reading corresponding to the diluted sample by 5.

After sample dilution, refer to *Table 1* to select volume and range.

Sample Seeding

Determining BOD of Seed
Certain types of BOD samples, such as many industrial discharges, do not contain sufficient bacteria to oxidize organic matter present in the sample. Some sewage treatment plant effluents are chlorinated to the extent that they are essentially sterile, making it impossible to perform a direct BOD test. To test such samples, seed each bottle with water known to contain an abundant bacterial population (e.g., domestic sewage or Polyseed Inoculum, Cat. No. 24712-00).

The BOD of the seed must be known in order to calculate the BOD of the sample. To determine the BOD of the seed, follow the same procedure used to determine the BOD of the sample. Run a BOD test on the seed and sample at the same time. For seed acclimation information, see *Seed Acclimatization on page 307*.

Determining Sample BOD

After determining the BOD of the seed, apply the following formula to determine the sample BOD.

$$\text{BOD sample} = \frac{\text{BOD observed} - (\text{Decimal fraction of seed used} \times \text{BOD seed})}{\text{Decimal fraction of sample used}}$$

Example:

A seeded sample is 10% seed and 90% sample (by volume). The observed BOD is 60 mg/L, and the pure seed BOD is 150 mg/L.

$$\text{BOD sample} = \frac{(60\ \text{mg/L}) - (0.10 \times 150\ \text{mg/L})}{0.90} = 50\ \text{mg/L}$$

Variations in Initial Bacterial Populations

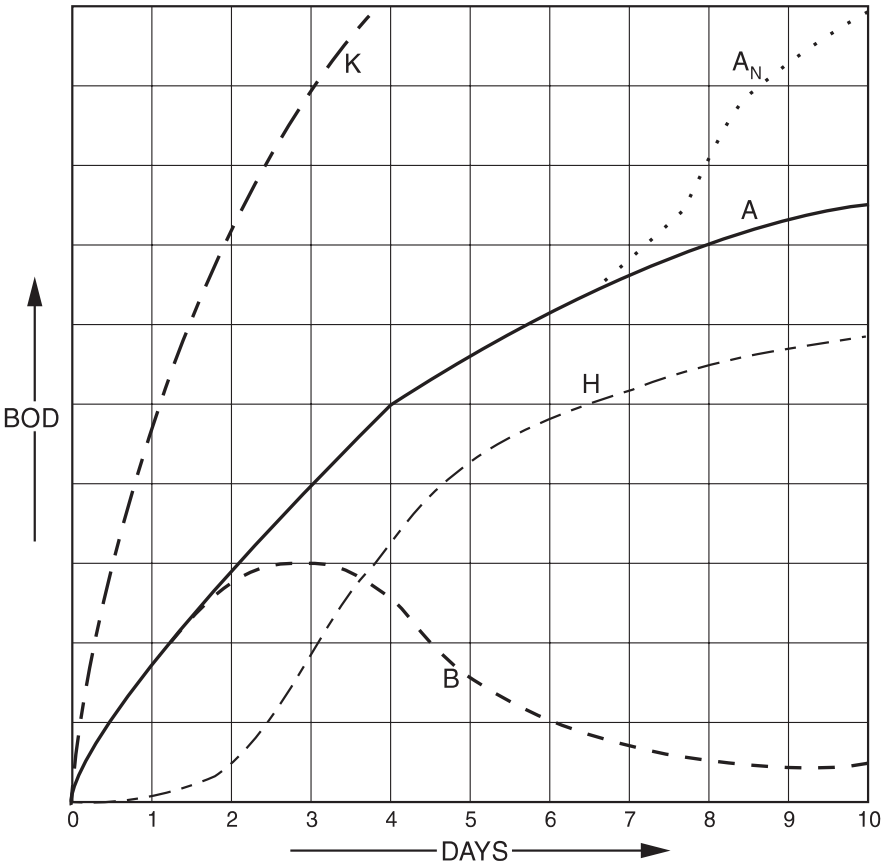
Low seed concentrations are more critical than those that are too high. They delay the start of oxidation and cause low BOD results. Use the trial and error method to determine the optimum concentration of seed for a specific waste material.

Choose the seed concentration yielding the highest corrected waste sample BOD. This seed percentage can range from 2–30%, depending on the waste material tested.

Interpreting Test Results

If the test proceeds correctly, the display should produce a curve similar to Curve A in *Figure 1*.

Figure 1 Example of BOD curves



If such a curve does not occur, one or more of the following problems may have occurred: bottle leak, time lag, high oxygen demand, or nitrification. These potential problems are discussed in detail in the next four sections.

Bottle Leak

A leak between the bottle cap and seal cup may cause readings similar to those plotted as Curve B in *Figure 1*, or may cause no response from the system. If such a response to BOD changes occurs, check for dirt inside the bottle cap and under the seal cup.

Time Lag

Tests that begin with insufficient bacteria during the incubation period produce data similar to that of Curve H in *Figure 1*. For sample processing when insufficient bacteria are present, seed the sample as described in *Sample Seeding* on page *301*.

Bacterial acclimation also produces conditions that could generate Curve H in *Figure 1*. This sometimes occurs when running standards, even though seed has been added.

High Oxygen Demand

Samples that are over range (for example, a BOD over 350 mg/L when a 160-mL sample is taken) will produce results as shown in Curve K in *Figure 1*. Dilute the sample according to the *Sample Dilutions* section, or use a higher BOD range and a different sample volume as directed in *Table 1*.

When the BOD range of a sample is unknown, use the results from the Chemical Oxygen Demand (COD) test, or the results from a series of BOD tests using the same sample but different volumes, or dilution ratios to select an appropriate BOD range. Generally, effluent normally is in the 0–70 mg/L range while influent is in the 0–700 mg/L range. When the BOD of the sample is greater than 700 mg/L, prepare a sample dilution (see *Sample Dilutions on page 300*).

Nitrification

The condition shown by Curve A_N in *Figure 1* is an example of nitrification. Biological oxidation of organic nitrogen usually occurs after five days with normal domestic waste because it takes that long for the nitrifying bacteria to develop; however, an abnormally high uptake of oxygen (especially when testing final effluent) is evidence of nitrifying bacteria adding appreciably to the oxygen demand.

Control nitrification problems with Hach Nitrification Inhibitor (Cat. No. 2533-35). Dispense the inhibitor powder into an empty sample bottle and then add the sample. When using the Hach Dispenser Cap (Cat No. 459-01), dispense two shots (approximately 0.16 grams) into the empty bottle.

Accuracy Check

To check test accuracy and proper performance of the BODTrak Apparatus, test a standard BOD sample and evaluate it for one or more of the mechanical, physical, or biological effects described in *Interpreting Test Results*.

Use a mixture of 150 mg/L each of glucose and glutamic acid as the BOD standard. A prepared BOD standard solution, 3000 mg/L each of glucose and glutamic acid, is available as the Voluette Ampule Standard for the Manometric Method (Cat. No. 14866-10). A 1:20 dilution of this standard (to 150 mg/L) is incorporated in the following procedure. Follow this procedure to analyze the 3000 mg/L prepared standard sample:

1. Shake three liters of distilled water in a partly filled container for one minute to saturate the water with oxygen.

2. Add the contents of one BOD Nutrient Buffer Pillow (Cat. No. 14861-98) for 3 L, and invert several times to mix.

3. Snap the neck off a Voluette Ampule Standard for BOD (Cat. No. 14866-10) and pipet 7 mL of standard into a sample bottle.

4. Add 133 mL of the nutrient buffer solution, prepared in step *2*, and 15 mL seed. A 10% by volume of seed in solution will result. See *Sample Seeding*.

5. Follow the general procedure for the BOD test using the 0–350 mg/L BOD range and a five-day test period.

6. Also perform a full strength BOD test on the pure seed to determine its BOD while determining the BOD of the standard.

7. To determine the BOD result, see *Determining Sample BOD*. The corrected BOD of the standard solution should be 198 ±30.5 mg/L.

Sample Temperature

The American Public Health Association (APHA) recommends a solution temperature of 20 ±1 °C (68 °F) for conducting the BOD test. Obtain this temperature by placing the BODTrak instrument

in an appropriate incubator and adjusting the temperature until the solution reaches 20 ±1 °C. An undercounter BOD Incubator and a combination BOD Incubator/Refrigerator are available (see *OPTIONAL APPARATUS*).

Samples should be cooled to incubation temperature. Seeding of samples with initially high sample temperatures may also be necessary because samples may have insufficient bacteria (see *Sample Seeding*). Determine the BOD of the seed and the BOD of the sample at the same time.

Other BOD Test Temperatures

The BOD test can be conducted at temperatures other than 20 °C. Tool's[1] results indicate that the five-day, 20 °C value can be obtained in 2.5 days at 35 °C. Middlebrooks[2] presents nomographs for converting BOD tests to temperatures other than 20 °C.

Interferences

Industrial and chlorinated samples often contain toxic substances and require special considerations when running BOD tests. The presence of toxic substances in the sample will cause decreased BOD values. Either remove the toxic substances or eliminate their effects by diluting the sample.

Chlorine

Low chlorine[3] concentrations may be dissipated by maintaining the sample at room temperature for 1–2 hours before testing. Remove the chlorine from samples with high chlorine levels by adding sodium thiosulfate as described below:

1. Add 10 mL of 0.02 N Sulfuric Acid Standard Solution and 10 mL of 100 mg/L Potassium Iodide Solution to a 100-mL portion of sample in a 250-mL Erlenmeyer flask.

1 H. R. Tool, Manometric Measurement of the Biochemical Oxygen Demand, *Water and Sewage Works Journal*, 114; 211-218, 1967.
2 E.J. Middlebrooks, A Nomograph for Solution of the BOD Equation, *Water and Sewage Works Journal*, 112; R230, 1965.
3 Measure chlorine with Hach Water Quality Chlorine Test Strips (Cat No. 27450-50).

2. Add three full droppers of Starch Indicator Solution and swirl to mix.

3. Titrate from dark blue to colorless with 0.025 N Sodium Thiosulfate Standard Solution.

4. Calculate the amount of Sodium Thiosulfate Standard Solution necessary to dechlorinate the remaining sample:

$$\text{mL of Sodium Thiosulfate} = \frac{(\text{mL used})(\text{mL sample to be dechlorinated})}{100}$$

5. Add the required amount of 0.025 N Sodium Thiosulfate Standard Solution to the sample and mix thoroughly. Wait 10 to 20 minutes before running the BOD test.

Other Toxic Materials

Determine the concentrations of other toxic materials such as phenols, heavy metals, and cyanides.

Dilute the sample with distilled water to eliminate the effect of these materials. The correct BOD is obtained when two successive dilutions result in the same sample BOD value. Or, the seed used in the dilution water may be acclimatized to tolerate such materials.

Seed Acclimatization

Domestic sewage or Polyseed Inoculum can provide seed for most samples. Polyseed Inoculum is ideally suited for domestic and industrial wastewater because it provides a constant seed source and is free of nitrifying microorganisms.

Pour the contents of one polyseed capsule into dilution water to rehydrate (refer to the procedure packaged with the Polyseed). Aerate and stir for one hour. Use enough of this solution so that it makes up 10 to 30% of the overall sample volume. The exact percentage of seed must be determined for each sample type.

For more information, *Standard Methods for the Examination of Water and Wastewater, 19th edition* emphasizes the importance of selecting the proper seed for specific wastes.

If the waste sample to be tested contains toxic materials such as phenol, formaldehyde, or other microbic inhibitory agents, use acclimated seed. Acclimate the seed in any non-metal or stainless steel gallon container fitted with an aeration system. Proceed as follows:

1. Aerate domestic sewage for about 24 hours. Allow one hour settling time for heavier materials to settle.

2. After the one hour settling, siphon and discard the top two-thirds of the volume.

3. Refill the container to the original volume with domestic sewage containing 10% of the waste material in question.

4. Repeat **steps 1–3**, increasing the addition of waste material by 10%. Stop the procedure when 100% waste material has been reached.

pH Effect

Low BOD test results occur when the pH of a test waste material exceeds the 6–8 range. The operator may maintain this pH to simulate original sample conditions or may adjust the pH to approach neutrality (buffered at pH 7). Neutralize samples containing caustic alkalinity or acidity by using 1.0 N (or weaker) sulfuric acid or sodium hydroxide, respectively.

Supersaturation

Reduce supersaturated cold samples (containing more than 9 mg/L dissolved oxygen at 20 °C) to saturation. To do so, first bring the sample temperature to about 20 °C. Then partly fill a sample bottle with sample and shake vigorously for two minutes, or aerate with filtered compressed air for two hours.

Summary of Method

Biochemical Oxygen Demand (BOD) is an empirical measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters.

BOD measures the amount of oxygen used by bacteria as they oxidize organic matter in the sample. The waste sample is put in an amber BOD bottle with an ample amount of air left above the sample. The bottle is connected to a pressure sensor. The bacteria use dissolved oxygen, which is replaced by the air above the sample. This causes a drop in air pressure in the bottle, which is registered by the pressure sensor. The drop can be read directly as mg/L BOD off the graphical display. Carbon dioxide produced by oxidation is removed by the lithium hydroxide crystals in the seal cup.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
BOD Nutrient Buffer Pillows, for preparing 300 mL	1	50/pkg	14160-66
BOD Nutrient Buffer Pillows, for preparing 3 L	1	50/pkg	14861-66
Grease, stopcock, tube	varies	75 g	562-75
Lithium Hydroxide, Powder Pillows	1	100/pkg	14163-69

REQUIRED APPARATUS

BODTrak™ Apparatus, 115/230V	1	each	26197-00
Bottle, BOD, amber	1	6/pkg	7144-21
Funnel, powder	1	each	22644-67
Seal cup	1	6/pkg	10977-52
Stir bar, magnetic	1	6/pkg	10764-16
Power cord, 115V	1	each	18010-00
Power cord, 230V	1	each	46836-00
Power Supply, 110/230 VAC	1	each	26249-00

OPTIONAL REAGENTS

Description	Unit	Cat. No.
BOD Nutrient Buffer Pillows, for preparing 6 L	50/pkg	14862-66
BOD Nutrient Buffer Pillows, for preparing 19 L	25/pkg	14863-98
Buffer Solution, phosphate type, pH 7.2	1000 mL	431-53
Calcium Chloride Solution	1 L	428-53
Ferric Chloride Solution	1 L	429-53
Magnesium Sulfate Solution	1 L	430-53
Chromic Acid Cleaning Solution	500 mL	1233-49
Nitrification Inhibitor	35 g	2533-35
Polyseed Inoculum	pkg/50	24712-00
Potassium Iodide Solution, 100 g/L	500 mL	12289-49
Potassium Permanganate	454 g	168-01
Sodium Hydroxide ACS Pellets	500 g	187-34
Sodium Hydroxide Standard Solution, 1.0 N	900 mL	1045-53
Sodium Sulfite Anhydrous, ACS	454 g	195-01
Sodium Thiosulfate Standard Solution, 0.025 N	1000 mL	352-53
Starch Indicator Solution	100 mL MDB	349-32
Sulfuric Acid, ACS:		
Concentrated	500 mL	979-49
0.02 N Standard Solution	1000 mL	203-53
1.0 N Standard Solution	1000 mL	1270-53
Voluette Ampule Standard for BOD, for manometric, 3000 mg/L10-mL ampule	16/pkg	14866-10
Water Quality Test strips, chlorine	50/pkg	27450-50

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Bottle, polyethylene, with spigot	4 L	14868-17
Brush, cylinder, size 2	each	687-00
Buret, straight stopcock, Teflon plug, 25 mL	each	14681-40
Clamp, buret, double	each	328-00
Cylinders, graduated:		
10 mL	each	508-38
25 mL	each	508-40
50 mL	each	508-41
100 mL	each	508-42
250 mL	each	508-46
500 mL	each	508-49
1000 mL	each	508-53
Dispenser Cap for 35g bottle (used with Nitrification Inhibitor)	each	459-01
Flask, Erlenmeyer, 500 mL	each	505-49
Flask, volumetric, 1000 mL	each	547-53
Incubator, under counter, Model 205, 120V	each	26162-00
Incubator, under counter, Model 205, 240V	each	26162-02
Incubator/Refrigerator, Model 207, 120V	each	26198-00
Incubator/Refrigerator, Model 207, 240V	each	26198-02
IncuTrol®/2, Temperature Regulator, 115V	each	2597-00
IncuTrol®/2, Temperature Regulator, 220V	each	2597-02
Pipet Filler	each	12189-00
Pipet, serological:		
1.0 mL	each	532-35
10 mL	each	532-38
Printer, 115 V, Epson Model LQ-570+ (graphic)	each	48236-00
Printer cable	each	26582-00
Sampler, sewage	each	427-00

OXYGEN DEMAND, CHEMICAL

Dichromate Reactor Digestion Method[1] (USEPA approved for wastewater analysis)[2]

Introduction

The Chemical Oxygen Demand (COD) test is used widely to estimate the amount of organic matter in wastewater. It is a measurement of the oxygen equivalent of the materials present in the wastewater that are subject to oxidation by a strong chemical oxidant, in this case dichromate. When wastewater contains only readily available organic bacterial food and no toxic matter, the COD test results provide a good estimate of BOD (Biochemical Oxygen Demand) values. In most real-world cases, COD has correlated well with COD values.

In the Dichromate Reactor Digestion Method test, the COD procedure is greatly simplified over the Dichromate Reflux Method. Small volumes of the water sample are pipetted into vials containing the premeasured reagents, including catalysts and chloride compensator. The vials are incubated until digestion is complete and then cooled. The COD measurement is made either with the spectrophotometer (accepted for reporting by the U.S. Environmental Protection Agency) or by titration.

Material Safety Data Sheets and Labels

Material Safety Data Sheets (MSDS) are supplied with all reagents. It is good laboratory practice to read the MSDS and the reagent container labels to familiarize yourself with the reagents used in this procedure.

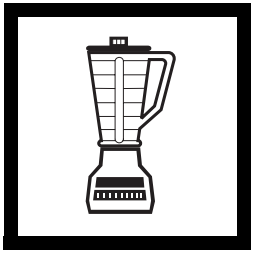
Analysis Procedure

Both the titrimetric measurement and the colorimetric measurement for determining Dichromate Chemical Oxygen Demand (COD) are detailed in this procedure. Sample digestion is required for both methods, with the choice of the final measurement method left to the analyst. Colorimetric measurement is the simpler and quicker of the two and is USEPA approved. Hach's titrimetric measurement should be used if turbidity or colored species remain after digestion; it is not USEPA approved.

1 Jirka, A.M.; Carter, M.J. *Analytical Chemistry*, 47(8), 1397, 1975.

2 Federal Register, April 21, 1980, 45(78), 26811-26812. The Ultra Low Range (0–40mg/L) COD Vials are **not** USEPA approved. High Range Plus (0–15000 mg/L) vials are **not** approved. The titrimetric measurement is **not** USEPA approved.

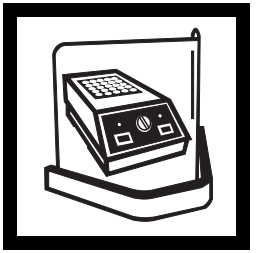
Digestion



1. Homogenize 100 mL of sample for 30 seconds in a blender.

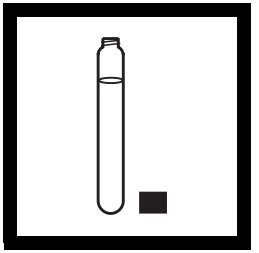
Note: *Mix the sample prior to homogenization. To improve accuracy and reproducibility pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate. For samples containing large amounts of solids, increase the homogenization time.*

Note: *Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if improperly handled. Please read all warnings and the safety section of this manual. Wear appropriate eye protection and clothing for adequate user protection. If contact occurs, flush the affected area with running water. Follow instructions carefully.*



2. Turn on the COD Reactor. Preheat to 150 °C. Place the plastic shield in front of the reactor.

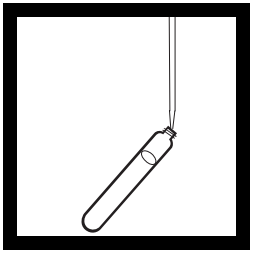
Note: *Ensure safety devices are in place to protect analyst from splattering should reagent leaking occur.*



3. Remove the cap of a COD Digestion Reagent Vial for the appropriate range:

Sample Conc. Range (mg/L)	COD Digestion Reagent Vial Type
0 to 40	Ultra Low Range
0 to 150	Low Range
0 to 1500	High Range
0 to 15000	High Range Plus

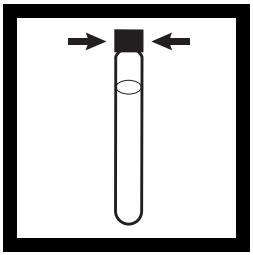
Note: *The reagent mixture is light-sensitive. Keep unused vials in the opaque shipping container, in a refrigerator if possible. The light striking the vials during the test will not affect results.*



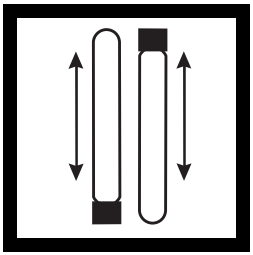
4. Hold the vial at a 45-degree angle. Pipet 2.00 mL (0.2 mL for the 0 to 15000 mg/L range) of sample into the vial.

Note: For the 0–15000 mg/L range, *pipet only 0.20 mL of sample, not 2.00 mL of sample, using a TenSette Pipet. For greater accuracy a minimum of three replicates should be analyzed and the results averaged.*

Note: *Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Do not run tests with vials which have been spilled. If spills occur, wash with running water.*

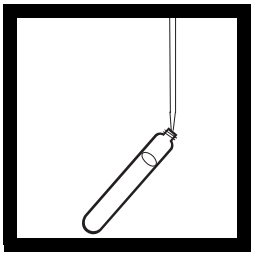


5. Replace the vial cap tightly. Rinse the outside of the COD vial with deionized water and wipe the vial clean with a paper towel.



6. Hold the vial by the cap and over a sink. Invert gently several times to mix the contents. Place the vial in the preheated COD Reactor.

Note: *The vial will become very hot during mixing.*



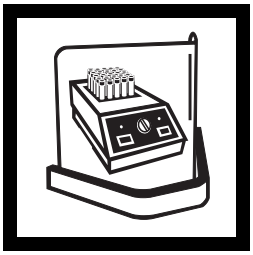
7. Prepare a blank by repeating *steps 3* to *6*, substituting 2.00 mL (0.2 mL for the 0 to 15000 mg/L range) deionized water for the sample.

Note: *Be sure the pipet is clean.*

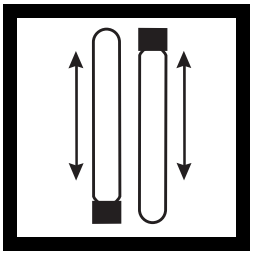
Note: *One blank must be run with each set of samples. Run samples and blanks with the same lot of vials.*

8. Heat the vials for 2 hours.

Note: *Many samples are digested completely in less than two hours. If desired, measure the concentration (while still hot) at 15-minute intervals until the reading remains unchanged. Cool the vials to room temperature for final measurement.*

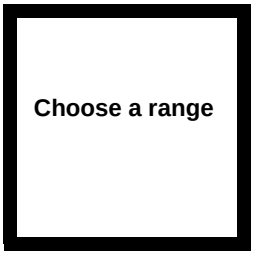


9. Turn the reactor off. Wait about 20 minutes for the vials to cool to 120 °C or less.



10. Invert each vial several times while still warm. Place the vials into a rack. Wait until the vials have cooled to room temperature.

Note: *If a pure green color appears in the reacted sample, measure the COD and, if necessary, repeat the test with a diluted sample.*



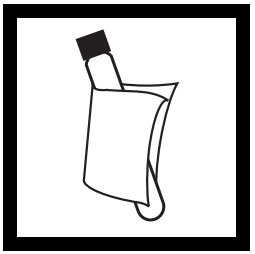
11. Use one of the following techniques to measure the COD:

- Colorimetric method, 0–40 mg/L COD
- Colorimetric method, 0–150 mg/L COD
- Colorimetric method, 0–1500 mg/L COD
- Colorimetric method, 0–15000 mg/L COD
- Titrimetric method, 0–150, 0-1500, 0–15000 mg/L COD

Colorimetric Measurement, 0 to 40 mg/L

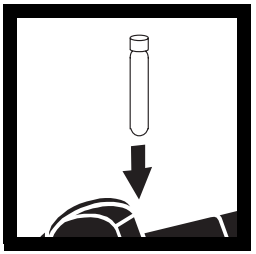
1. Place the COD/TNT Vial Adapter into the instrument cell holder.

Note: *The COD/TNT Adapter is NOT designed to allow readings on hot vials (150 °C).*



2. Clean the outside of the vials with a towel.

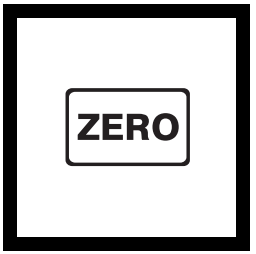
Note: *Wiping with a damp towel, followed by a dry one removes fingerprints or other marks.*



3. Place the blank into the adapter with the Hach logo facing the front of the instrument. Close the light shield.

Note: *Preparation of the blank is described in the digestion procedure.*

Note: *The blank is stable when stored in the dark; see* Blanks For Colorimetric Measurement *following these steps.*

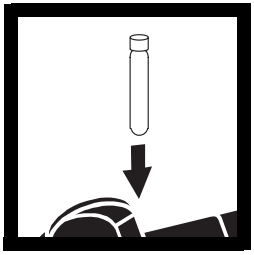


4. Zero the instrument with the blank, using the settings below.

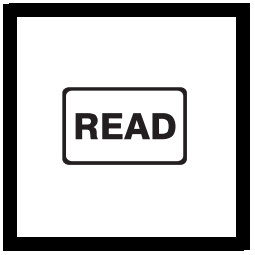
DR/800s
Program No. NA

DR/2010
Program No. NA
DR/4000
Program No. 2700
350 nm

Note: *Ultra Low Range Vials may be used only with spectrophotometers with 350-nm capability*



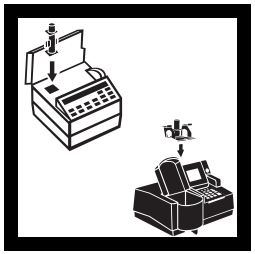
5. Place the sample into the adapter with the Hach logo facing the front of the instrument. Close the light shield.



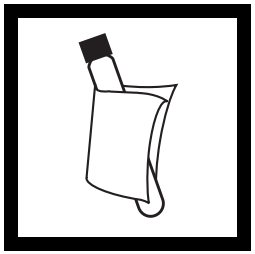
6. Read the mg/L COD

Note: *If the display shows 45 mg/L COD or greater, repeat the test with a diluted sample or use a Low Range or High Range COD Reagent Vial.*

Colorimetric Measurement, 0 to 150 mg/L COD

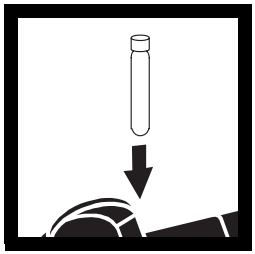


1. Place the COD/TNT Vial Adapter into the cell holder.



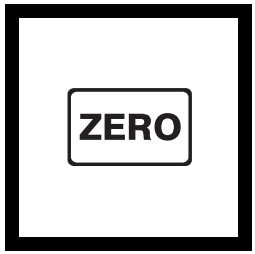
2. Clean the outside of the vials with a towel.

Note: *Wiping with a damp towel, followed by a dry one, removes fingerprints or other marks.*



3. Place the blank into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

Note: *The blank is stable when stored in the dark; see Blanks for Colorimetric Determination following these procedures.*

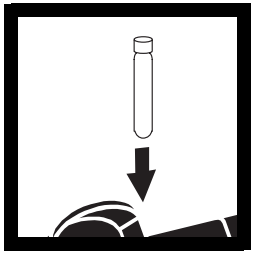


4. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 16

DR/2010
Program No. 430
420 nm

DR/4000
Program No. 2710
420 nm

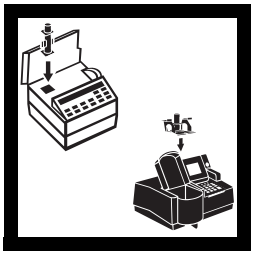


5. Place the sample into the adapter with the Hach logo facing the front of the instrument. Close the light shield.

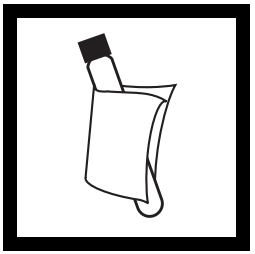
6. Read the mg/L COD.

Note: *For most accurate results with samples near 150 mg/L, repeat the test with a diluted sample.*

Colorimetric Measurement, 0 to 1500 and 0 to 15000 mg/L

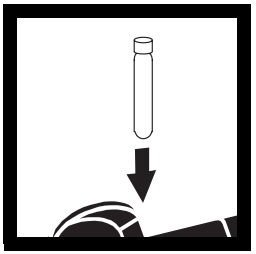


1. Place the COD/ TNT Vial Adapter into the cell holder.



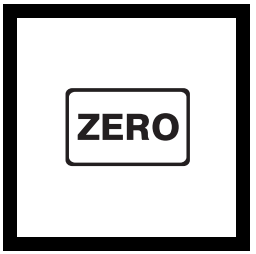
2. Clean the outside of the vials with a towel.

Note: *Wiping with a damp towel followed by a dry one removes fingerprints or other marks.*



3. Place the blank into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

Note: *The blank is stable when stored in the dark. See Blanks for Colorimetric Measurement following these procedures.*

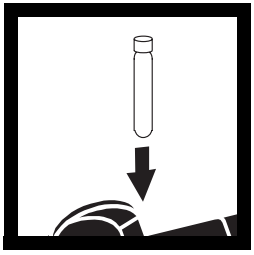


4. Zero the instrument with the blank, using the settings below.

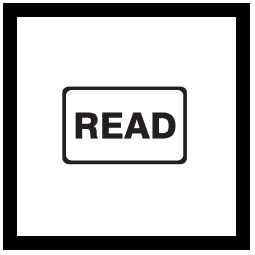
DR/800s
Program No. 17

DR/2010
Program No. 435
620 nm

DR/4000
Program No. 2720
620 nm



5. Place the sample into the adapter with the Hach logo facing the front of the instrument. Close the light shield.

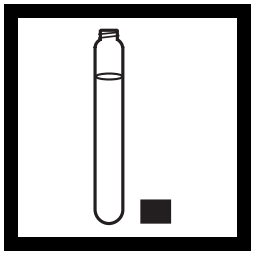


6. Read the mg/L COD.

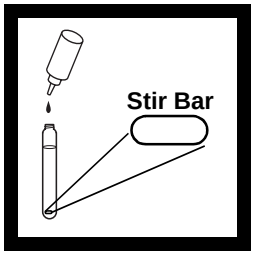
Note: *For most accurate results with samples near 1500 or 15,000mg/L, repeat the test with a diluted sample.*

Note: *When using High Range Plus Vials, multiply the reading by 10.*

Titrametric Measurement, 0-150, 0-1500 and 0-15000 mg/L COD

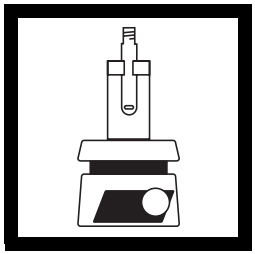


1. Carefully remove the cap of a digested vial. Rinse the inside walls with less than 1 mL of deionized water.

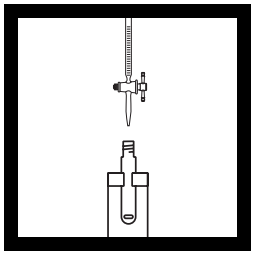


2. Add a small Teflon-coated stirring bar and one drop of the appropriate Ferroin Indicator Solution. When using the Low Range COD Digestion Vials, use Low Range Ferroin Indicator Solution. When using the High Range or High Range Plus COD Digestion Reagent Vials, use High Range Ferroin Indicator Solution.

Note: *If the color of the prepared sample changes from blue-green to orange-brown, the COD value is out of range. Dilute the sample and repeat the digestion.*



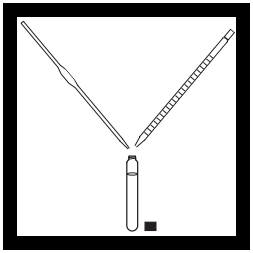
3. Place the vial on the titration stand. Turn on the magnetic stirrer.

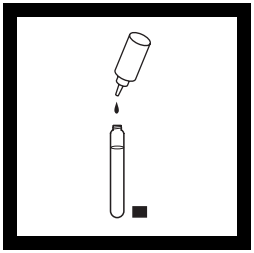


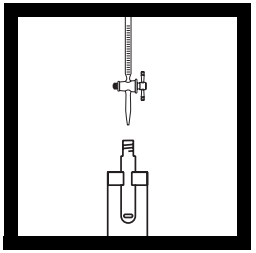
4. Titrate with the appropriate Ferrous Ammonium Sulfate Standard Solution (FAS) until the sample color changes sharply from greenish-blue to orange-brown. When using the Low Range COD Digestion Reagent Vials, use 0.0125N FAS. When using the High Range or High Range Plus COD Digestion Reagent Vials, use 0.125N FAS. Record the mL of titrant required. The mL required for the prepared sample is value B. The mL required for the blank is value A.

Note: *Mix the FAS bottle well before using.*

Note: *Values A and B are used in step 8.*







$$(A - B) \times \frac{2000}{C} \times M = \text{mg/L COD}$$

5. Pipet 2.00 mL of Potassium Dichromate Standard Solution into an empty vial. When using the Low Range COD Digestion Reagent Vials, use a 0.025 N solution. When using High Range or High Range Plus COD Digestion Reagent Vials, use a 0.25 N solution.

Add 3 ml of sulfuric acid to the vial. Swirl to mix. Wait for the solution to cool until the vial is comfortable to touch.

Note: Steps 5 *through* 7 *need only be done daily because the FAS deteriorates over time.*

6. Add one drop of the Ferroin Indicator Solution selected in *step 2.*

Add a stir bar and titrate with the Ferrous Ammonium Sulfate Standard Solution selected in *step 4* until the color changes from greenish-blue to orange-brown. Record the number of mL required. This is value C in the following equation.

Note: *Mix the FAS bottle well before using.*

Note: *To remove the stir bar from the vial, tip the vial at an angle in one hand and hold the stir bar retriever in the other. Place the retriever near the bottom of the vial on the OUTSIDE. Move the retriever up the wall to the top of the vial.*

7. Determine the mg/L COD according to the following equation:

$$(A - B) \times \frac{2000}{C} \times M = \text{mg/L COD}$$

Where:

A = mL used in titration of reagent blank

B = mL used in titration of prepared sample

C = mL used in titration of standard solution in step 7 above

M = 0.1 when using Low Range Digestion Vials

M = 1 when using High Range Digestion Vials

M = 10 when using High Range Plus Digestion Vials

For example, when using Low Range COD Reagent Vials:

A= 3.95 mL
B = 2.00 mL
C = 4.00 mL
M = 0.1

mg/L COD =

$$(3.95 - 2.0) \times \frac{2000}{4.00} \times 0.1 = 97.5$$

Sampling and Storage

Collect samples in glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days. When significant amounts of preservatives are used, a volume correction should be made for the extra acid by dividing the total volume (sample + acid) by the sample volume and multiplying this value by the final test reading.

Accuracy Check

Standard Solution Method

Check the accuracy of the 0 to 40 mg/L range with a 30 mg/L COD standard solution. Using Class A glassware, prepare a 1000-mg/L solution by diluting 0.85 g of dried (120 °C, overnight) potassium acid phthalate (KHP) in 1000 mL of organic-free deionized water. Prepare a 30 mg/L dilution by diluting 3.00 mL of this solution into a 100.0 mL volumetric flask. Dilute to volume with deionized water, stopper, and invert 10 times to mix.

Check the accuracy of the 0 to 150 mg/L range with a 100 mg/L standard. Prepare by dissolving 0.85 g of dried (120 °C, overnight) potassium acid phthalate (KHP) in one liter of deionized water. Use 2 mL as the sample volume. The expected result will be 100 mg/L COD. Or, dilute 10 mL of 1000-mg/L COD Standard Solution to 100 mL to produce a 100-mg/L standard.

Check the accuracy of the 0 to 1500 mg/L range by using either a 300 mg/L or 1000 mg/L COD Standard Solution. Use 2 mL of one of these solutions as the sample volume; the expected result will be 300 or 1000 mg/L COD respectively.

Or, prepare a 500 mg/L standard by dissolving 0.425 g of dried (120 °C, overnight) KHP. Dilute to one liter with deionized water.

Check the accuracy of the 0 to 15000 mg/L range by using a 10,000 mg/L COD standard solution. Prepare the 10,000 mg/L

solution by dissolving 8.500 g of dried (120 °C, overnight) KHP in one liter of deionized water. Use 0.2 mL of this solution as the sample volume; the expected result will be 10,000 mg/L COD.

Preparing Organic-Free Water

To prepare organic-free water with no measurable COD:

1. Pour 1.0 liter of deionized water with low COD in a 2-liter Erlenmeyer flask.
2. Add the contents of one Potassium Persulfate Powder Pillow to the flask. Swirl to dissolve.
3. Suspend a UV lamp in the flask so the glass portion of the bulb is immersed and the black bakelite portion is above the solution. Follow the safety and operation instructions recommended in the UV lamp kit. Safety UV goggles should be worn for eye protection.
4. Irradiate the solution with UV light for at least two hours (overnight is fine).
5. Remove the lamp from the solution. Add one level 0.05-gram scoop of Nickel Sulfate to the solution.
6. Heat the water to a boil. Remove the flask from the hot plate and cover it with a watch glass.
7. Let the flask cools to room temperature. The water will have zero oxygen demand. Seal the flask top with aluminum foil to prevent organic contamination. The water should stay free of oxygen demand for one week if properly sealed.

Blanks for Colorimetric Measurement

The blank may be used repeatedly for measurements using the same lot of vials. Store it in the dark. Monitor decomposition by measuring the absorbance at the appropriate wavelength 350, 420, or 620 nm.) Zero the instrument in the absorbance mode, using a culture tube (see Optional Apparatus) containing 5 mL of deionized water. Measure the absorbance of the blank and record the value. Prepare a blank when the absorbance has changed by approximately 0.010 absorbance units.

Interferences

Bromide interference will not be controlled by mercuric sulfate.

Chloride[1] is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in column 1 in the table below. Samples with higher chloride concentrations should be diluted. Dilute the sample enough to reduce the chloride concentration to the level given in column 2.

If sample dilution will cause the COD concentration to be too low for accurate determination, add 0.50 g of mercuric sulfate ($HgSO_4$) to each COD vial before the sample is added. The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in column 3.

Vial Type Used	Maximum Cl- concentration in sample (mg/L)	Suggested Cl- concentration of diluted sample (mg/L)	Maximum Cl^- concentration in sample with 0.5 g $HgSO_4$ Added (mg/L)
Ultra Low Range	2000	1000	NA
Low Range	2000	1000	8000
High Range	2000	1000	4000
Ultra High Range	20000	10000	40000

Waste Management

Final samples will contain mercury, silver, and chromium at concentration levels regulated as hazardous waste by the Federal RCRA. Contact your governing local, state, or federal agency for further information on proper disposal of these materials.

Summary of Method

The mg/L COD results are defined as the mg of O_2 consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}). When the 0–40 mg/L or 0–150 mg/L colorimetric measurement is used, the amount of Cr^{6+} remaining is determined. When the 0–1500 mg/L or 0–15000 mg/L

1 Measure chloride with Hach Water Quality Test Strips, High Range Chloride (Cat. No. 27513-40).

colorimetric measurement is used, the amount of Cr^{3+} produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences.

REQUIRED REAGENTS (for colorimetric measurement)

Description	Quantity Required Per Test	Unit	Cat. No.
Select the appropriate COD Digestion Reagent Vial:			
Ultra Low Range, 0 to 40 mg/L COD	1 to 2	25/pkg	24158-25
Low Range, 0 to 150 mg/L COD	1 to 2	25/pkg	21258-25
High Range, 0 to 1500 mg/L COD	1 to 2	25/pkg	21259-25
High Range Plus, 0 to 15 000 mg/L COD	1 to 2	25/pkg	24159-25
Water, deionized	varies	4 L	272-56

REQUIRED REAGENTS (for titrimetric measurement)**

Pick one or both Potassium Dichromate Standard Solutions:			
0.025 N	2 mL	500 mL	164-49
0.25 N	2 mL	1000 mL	1809-53
Sulfuric Acid, ACS	3 mL	500 mL[1]	979-49
Water, Deionized	varies	4 L	272-56
Pick the appropriate COD Digestion Reagent Vial:			
Low Range	1-2	25/pkg*	21258-25
High Range	1-2	25/pkg*	21259-25
High Range Plus	1-2	25/pkg	24159-25
Pick one or both Ferroin Indicator Solutions:			
Low Range	1-2 drops	29 mL DB	20551-33
High Range	1-2 drops	29 mL DB	1812-33
Pick one or both Ferrous Ammonium Sulfate Standard Solutions:[2]			
0.0125 N	varies	1000 mL	14237-53
0.125 N	varies	500 mL	20548-49

1 Contact Hach for larger sizes.

2 Ferrous ammonium sulfate standard solutions, as prepared by Hach, have cadmium pellets in each bottle. The cadmium pellets help preserve the standard solution. Before filling the buret, the bottle should be swirled to bring the upper layer of solution in contact with the pellets. When titrating these solutions, do not return unused portions from the buret to the bottle or allow solution to stand in the buret for long periods of time. Do NOT use an automatic buret with a reservoir that holds more solution than can be used in one day.

REQUIRED APPARATUS (for colorimetric measurement)

Description	Quantity Required Per Test	Unit	Cat. No
Cap Tool, COD (for High Range Plus vials)	1	each	45587-00
COD Reactor, 120/240 Vac, North American fuses and plug	1	each	45600-00
COD Reactor, 230 Vac, 50 Hz, European fuses and power cord	1	each	45600-02
COD Test Tube Adapter, DR/4000	1	each	48189-00
COD Vial Adapter, DR/2010	1	each	44799-00
COD/TNT Adapter, DR/800	1	each	48464-00
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	varies	50/pkg*	21856-96
Pipet, volumetric, Class A, 2.00 mL	1	each	14515-36
Pipet Filler, safety bulb	1	each	14651-00
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1 to 2	each	18641-00

REQUIRED APPARATUS (for titrimetric measurement)

Bottle, wash, 500 mL	1	each	620-11
Buret Clamp, double	1	each	328-00
Buret, automatic, Class A, 5.00 mL	1	each	20550-37
COD Reactor, 120/240 Vac, North American fuses and plug	1	each	45600-00
COD Reactor, 120/240 Vac with European fuses and power cord	1	each	45600-02
Pipet, volumetric, Class A, 2.00 mL	1	each	14515-36
Pipet, Mohr, 5.00 mL	1	each	20934-37
Pipet filler, safety bulb	1	each	14651-00
Safety Shield, laboratory bench	1	each	50030-00
Stir Bar	1	each	20549-59
Stir Bar Retriever	1	each	15232-00
Stirrer, magnetic, 120 Vac, 50/60 Hz	1	each	23444-00
Stirrer, magnetic, 240 Vac, 50/60 Hz	1	each	23444-02
Support Stand	1	each	563-00
Test Tube Rack, 8 place (2 recommended)	1	each	18641-00
Titration Stand, test tube	1	each	18642-00

OPTIONAL REAGENTS

Description	Unit	Cat. No.
COD Digestion Reagent Vials, 0 to 40 mg/L COD	150/pkg	24158-15
COD Digestion Reagent Vials, 0 to 150 mg/L COD	150/pkg	21258-15
COD Digestion Reagent Vials, 0 to 1500 mg/L COD	150/pkg	21259-15
COD Standard Solution, 300 mg/L	200 mL	12186-29
COD Standard Solution, 1000 mg/L	200 mL	22539-29
Mercuric Sulfate, ACS	28.3 grams	1915-20
Nickel Sulfate, ACS	25 grams	11264-24
Potassium Acid Phthalate, ACS	500 g	315-34
Sulfuric Acid, ACS	500 mL[1]	979-49
Potassium Persulfate Powder Pillows	100/pkg	20847-69
Water Quality Test Strips for High Range Chloride	40/pkg	27513-40

OPTIONAL APPARATUS

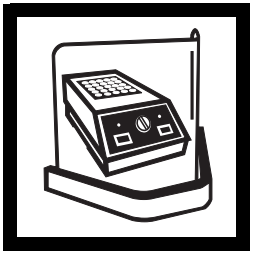
Balance, analytical, 115 V	each	26103-00
Balance, analytical, 230 V	each	26103-02
Beaker, 250 mL	each	500-46
Blender, 120 Vac, 1.2-L, 2-speed	each	26161-00
Blender, 240 Vac, 1.2-L, 2-speed	each	26161-02
Culture Tube, 16 x 100	each	22758-00
Culture Tube Cap	each	22411-00
Cylinder, graduated, 5 mL	each	508-37
Electromagnetic Stirrer, 120 V, with electrode stand	each	45300-01
Electromagnetic Stirrer, 230 V, with electrode standS	each	45300-02
Flask, volumetric, Class A, 1000 mL	each	14574-53
Flask, volumetric, Class A, 100 mL	each	14574-42
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipet, serological, 5 mL	each	532-37
Pipet, volumetric, Class A, 10 mL	each	14515-38
Spoon, measuring, 0.5 g	each	907-00
Stir Bar, 22.2 x 4.76 mm ($^{7}/_{8}$ x $^{3}/_{16}$ in.)	each	45315-00
Stir Bar Retriever	each	15232-00
UV Lamp Kit, 115 Vac	each	20828-00
UV Lamp Kit, 230 Vac	each	20828-02

1 Contact Hach for larger sizes.

OXYGEN DEMAND, CHEMICAL

Manganese III Reactor Digestion Method (without chloride removal)*
Range: 30 to 18000 mg/L (may require dilution)

The Manganese III COD method is an alternative to the dichromate COD methods. The Manganese III COD reagent does **not** contain metals that are regulated for disposal under RCRA, and neither do the Chloride Removal Cartridges used in the method that requires chloride removal. The results of both methods are similar. Results of the Manganese III COD method show a good correlation to results from the dichromate COD and five-day BOD methods. Use this method when proper disposal is not available for dichromate COD vials or disposal is too expensive. This method is not approved by the USEPA.

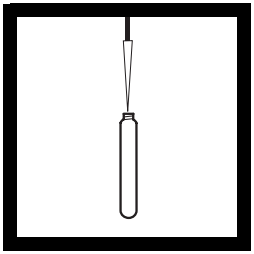


1. Turn on the COD Reactor and heat to 150 °C. Place the shield in front of the reactor.

Note: *To determine if the sample contains chloride, use Water Quality Test Strips for Low range Chloride. If the sample contains chloride use the method that removes chloride form the sample (follows this method).*

2. Homogenize 100 mL of sample for 30 seconds in a blender.

Note: *Continue mixing the sample while pipetting if suspended solids are present. Blending promotes even distribution of solids and improves accuracy and reproducibility.*



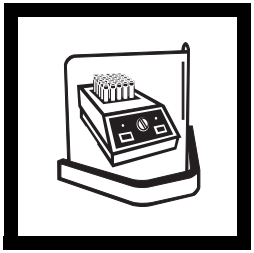
3. Pipet 0.50 ml of homogenized sample into a Mn III COD vial. Cap and invert several times to mix.

Note: *If the sample COD value is not between 30–1000 mg/L, dilute the sample with deionized water to obtain this range. Multiply the final result by the dilution factor.*

4. Prepare a blank by substituting 0.50 mL of deionized water for the sample.

Note: *The reagent blank is stable and can be reused. Verify reagent blank quality by measuring the absorbance of the blank vs. a clean COD vial filled with deionized water. The absorbance range should be about 1.4–1.5.*

* U.S. Patent 5,556,787 on method.

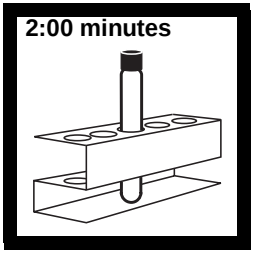


5. Place the vials in the COD Reactor that is preheated to 150 °C. Digest for one hour.

Note: *Boiling sample in the vials during digestion indicates the vial is not properly sealed; test results will be invalid.*

Note: *Samples can be digested up to four hours to oxidize more resistant organics. Treat the prepared blank in the same manner.*

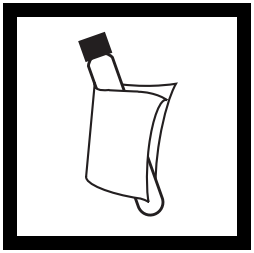
Note: *Ensure safety devices are in place to protect the analyst from splattering if leaks occur. Spilled reagent will affect test accuracy and is hazardous. Do not run tests with vials which have been spilled.*



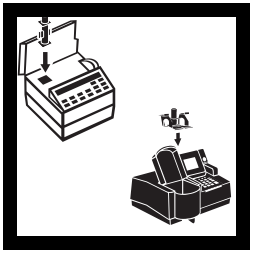
6. Remove the vials and place them in a cooling rack for two minutes to air cool. Then cool the vials to room temperature in a cool water bath or running tap water. This usually takes about three minutes.

Note: *Occasionally a vial will develop a colorless upper layer and a purple lower layer. Invert the vial several times to mix and proceed.*

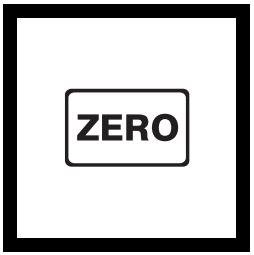
Note: *The Hach COD Vial Lifter allows the transfer of several vials at once.*



7. Remove the vials from the water and wipe with a clean, dry paper towel. Invert the vials several times to mix.



8. Place the COD Vial Adapter into the cell holder.

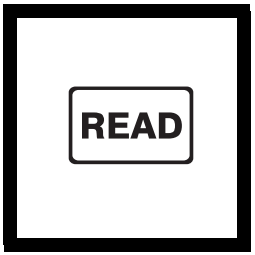


9. Place the blank in the instrument. Zero the instrument using the settings below.

DR/800s
Program No. 18

DR/2010
Program No. 432
510 nm

DR/4000
Program No. 2730
510 nm



10. Place the sample in the sample cell adapter. Place the cover on the adapter. Read the mg/L COD.

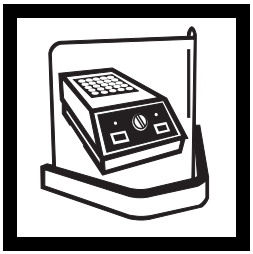
Note: *Adjust the results for any sample dilution in step 2.*

Additional Information

For information about Sampling and Storage, Accuracy Checks, Interferences, Summary of Method, Reagents and Apparatus, see the following procedure.

OXYGEN DEMAND, CHEMICAL

Manganese III Reactor Digestion Method (with chloride removal)*
Range: 20 to 18000 mg/L

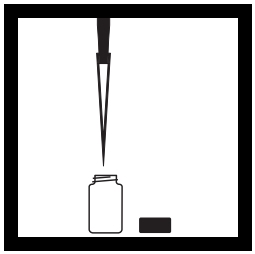


1. Turn on the COD Reactor and heat to 150 °C while preparing the sample and blank.

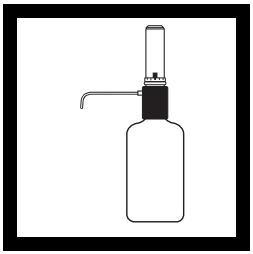
2. Homogenize 100 mL of sample for 30 seconds in a blender.

Note: *Blending promotes even distribution of solids and improves accuracy and reproducibility.*

Note: *If suspended solids are present, continue mixing the sample while pipetting.*



3. Using a TenSette Pipet or a pipet and safety bulb, pipet 9.0 mL of homogenized sample into an empty glass mixing cell. If the sample COD exceeds 1000 mg/L, dilute the sample as described in *Table 1* on page *332*.



4. Using an automatic dispenser or TenSette Pipet, add 1.0 mL of concentrated sulfuric acid to the mixing cell.

Note: *Mixing concentrated sulfuric acid and water does not give a volume addition. Adding 1.0 mL of concentrated sulfuric acid to 9.0 mL of sample does not result in a final volume of 10.0 mL. This factor is built into the calibration curve.*

Caution:
Some of the chemicals and apparatus used in this procedure may be hazardous if inappropriately handled or accidentally misused. Wear appropriate eye protection and clothing. If contact occurs, wash the affected area with running water. Follow all directions carefully.

* U.S. Patent 5,556,787 on method. U.S. patents 5,667,754 and 5,683,914 for Chloride Removal Cartridge. Patent pending on Vacuum Pretreatment Device.

Table 1 Dilution Table

Sample (mL)	Deionized Water (mL)	Range (mg/L COD)	Multiplication Factor
6.0	3.0	30-1500	1.5
3.0	6.0	60-3000	3
1.0	8.0	180-9000	9
0.5	8.5	360-18000	18

All dilutions require that the ratio of sample to sulfuric acid remain at 9:1. For other dilutions that are not listed in *Table 1*, simply add the sample volume + deionized water and divide by the sample volume to obtain the multiplication factor.

Example:

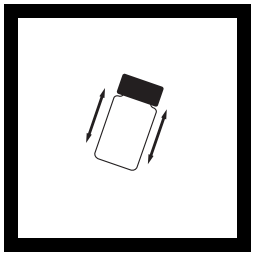
Dilute the sample to a range of 90–4500 mg/L COD

Sample Volume (2.0 mL) + Demineralized water (7.0 mL) = Total Volume (9.0 mL)

$$\text{Multiplication Factor} = \frac{\text{Total Volume}}{\text{Sample Volume}} = \frac{9.0\ \text{mL}}{2.0\ \text{mL}} = 4.5$$

Standard test range is 50-1000 mg/L COD
Example Test Range = 4.5 (50) to 4.5 (1000) = 225-4500 mg/L COD

It is best to use 0.5 mL or more of sample for diluting. If sample values exceed 18,000 mg/L COD, use a separate sample dilution before the sample chloride removal procedure.



5. Cap the cell tightly and invert it several times. The solution will become hot. Cool to room temperature before proceeding.

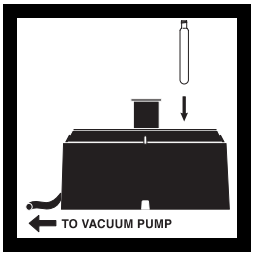
Note: *Acidified samples are stable for several months when refrigerated at 4 °C.*

6. Prepare a blank by repeating *steps 3-5*, substituting 9.0 mL of deionized water for the sample.

Note: *The reagent blank is stable and can be reused. Verify reagent blank quality by measuring the absorbance of the blank vs. a clean COD vial filled with deionized water. The absorbance range, when using chloride removal, should be about 1.4–1.5.*

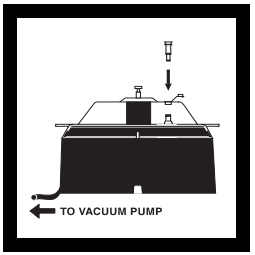
Note: *Use a clean pipet or rinse it thoroughly.*

Note: *Prepare one blank for each lot of reagents (lot number is on the container label).*

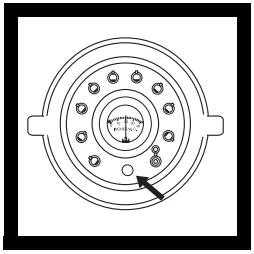


7. Label each Mn III COD vial and remove the cap. Place the vial in one of the numbered holes in the Vacuum Pretreatment Device (VPD) base.

Note: *The VPD must be attached to a vacuum pump (not an aspirator-type vacuum) that can create a vacuum of 20 to 25 inches of mercury.*

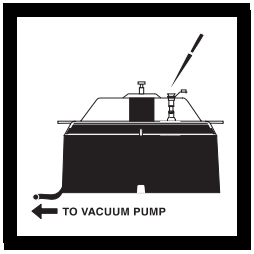


8. Place the VPD top on the base. Insert a fresh Chloride Removal Cartridge (CRC) directly above each Mn III COD Reagent Vial. Be sure the CRC filter is laying flat on the bottom grid of the sample cavity. Plug any open holes in the VPD top using the stoppers provided.



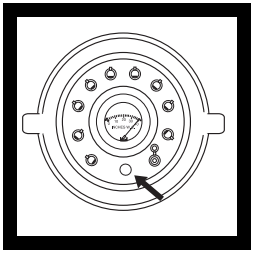
9. Turn the vacuum pump on and adjust the vacuum regulator valve on top of the VPD until the internal gauge reads 20 inches of water.

Note: *The optimum setting allows the sample to flow through the CRC in about 30 to 45 seconds.*



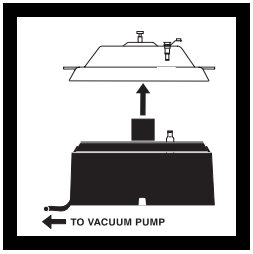
10. Pipet 0.60 mL of acidified sample (made in steps 3–5) into the CRC. Pipet 0.60 mL of acidified blank into another CRC.

Note: *If the sample does not flow through the CRC, increase the vacuum until flow starts, then reduce the vacuum to 20 inches of water and proceed.*

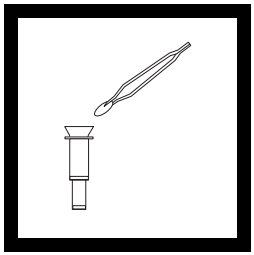


11. Close the vacuum regulator valve completely to achieve full vacuum. After one minute under full vacuum, open the VPD vacuum regulator valve to release the vacuum.

Note: *The maximum range of the vacuum gauge in the VPD is 40 inches of water; the gauge will not indicate the full vacuum level obtained. Full vacuum is 20–25 inches of mercury; this can be measured at the vacuum pump with a gauge that is calibrated in inches of mercury.*



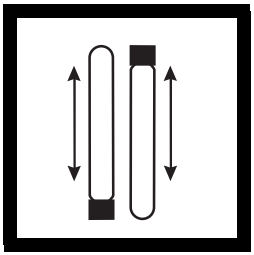
12. Turn the pump off. Remove the VPD top and set it beside the base.



13. Use forceps to remove the filter from the top of each CRC. Place each filter in the corresponding Mn III COD Vial (use the numbers on the VPD as a guide).

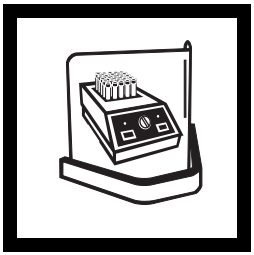
Note: *To remove the CRC filter, slide the forceps tip down the inside wall of the cartridge and grasp the edge of the filter. Carefully withdraw the filter, being careful not to lose any sample solids.*

Note: *Clean forcep tips between samples by wiping with a clean towel or rinsing with deionized water.*



14. Remove the Mn III COD vial from the vacuum chamber and replace the original cap. Screw the cap on tightly. Invert several times to mix.

Note: *Dispose of the used Chloride Removal Cartridge. Do not reuse it.*

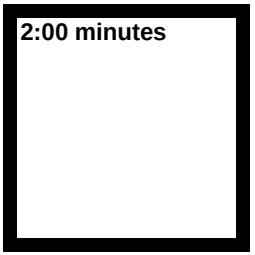


15. Place the vials in the COD Reactor that has been preheated to 150 °C. Place the safety shield in front of the reactor. Digest for one hour.

Note: *Boiling sample in the vials indicates the vial is not properly sealed; test results will be invalid.*

Note: *Samples can be digested up to 4 hours to oxidize more resistant organics. Treat the prepared blank in the same manner.*

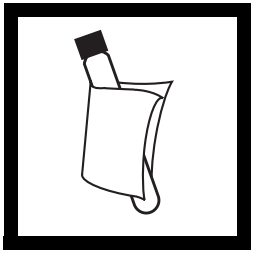
Note: *Ensure safety devices are in place to protect against splattering if leaks occur. Leaked reagent will affect test results and is hazardous. Do not continue testing with these vials.*



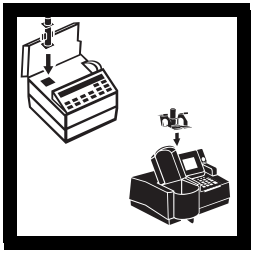
16. Remove the vials and place them in a cooling rack for two minutes to air cool. Then cool the vials to room temperature in a cool water bath or running tap water. This usually takes about three minutes.

Note: *Occasionally a vial will develop a colorless upper layer and a purple lower layer. Invert the vial several times to mix and proceed.*

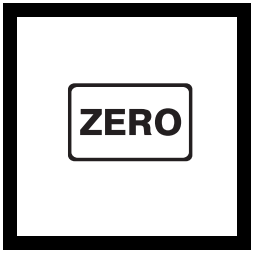
Note: *Use the Hach COD Vial Lifter to transfer several vials at once.*



17. Remove the vials from the water and wipe dry with a clean paper towel. Invert the vials several times to mix.



18. Place the COD Vial Adapter into the cell holder.

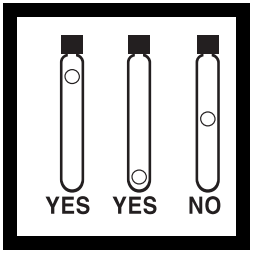


19. Place the blank in the instrument. Zero the instrument using the settings below.

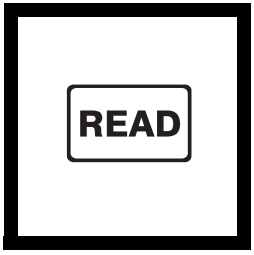
DR/800s
Program No. 18

DR/2010
Program No. 432
510 nm

DR/4000
Program No. 2730
510 nm



20. Make sure the filter disc is not suspended in the middle of the vial; it can interfere with the instrument reading. Move it with gentle swirling or by lightly tapping the vial on the table top.



21. Place the sample in the sample cell adapter. Place the cover on the adapter. Read the mg/L COD.

Note: *Adjust the results for any sample dilution in Table 1.*

Sampling and Storage

Collect samples in clean glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples adjusted with concentrated sulfuric acid to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days. Correct results for volume additions.

Accuracy Check

Standard Solution Method

Prepare an 800-mg/L COD standard solution by adding 0.6808 g of dried (103 °C, overnight) potassium acid phthalate (KHP) to one liter of deionized water. Use 0.50 mL of this solution (0.60 mL for the chloride removal procedure) as the sample volume. The result should be 800 ±26 mg/L COD. An 800-mg/L COD solution can also be purchased from Hach (see *Optional Reagents*).

Interferences

Inorganic materials may be oxidized by trivalent manganese and cause a positive interference if present in significant amounts. Chloride*, the most common interference, is removed by sample pretreatment with the Chloride Removal Cartridge. If chloride is absent or present in insignificant levels, the pretreatment can be omitted. To determine if chloride will affect test results, run routine samples with and without the chloride removal, then compare results. Other inorganic interferences (i.e., nitrite, ferrous iron, sulfide) are not usually present in significant amounts. If necessary, the analyst can correct for these interferences after determining their concentrations with separate methods and subtracting the interference value from the COD test results. Ammonia nitrogen is known to interfere in the presence of chloride; it does not interfere if chloride is absent.

* Measure chloride with Hach Quantab titrators for Low range chloride (27449-40).

Summary of Method

Chemical oxygen demand (COD) is "... a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant" (APHA Standard Methods, 19th ed., 1995). Trivalent manganese is a strong, non-carcinogenic chemical oxidant that changes quantitatively from purple to colorless when it reacts with organic matter. It typically oxidizes about 80% of the organic compounds. Studies have shown that the reactions are highly reproducible and test results correlate closely to Biochemical Oxygen Demand (BOD) values and hexavalent chromium COD tests. None of the oxygen demand tests oxidize100% of all organic compounds.

A calibration is provided which is based on the oxidation of Potassium Acid Phthalate (KHP). Response may vary with different wastewaters. The KHP calibration is adequate for most applications. The highest degree of accuracy is obtained when test results are correlated to a standard reference method such as BOD or one of the chromium COD methods. Special waste streams or classes will require a separate calibration to obtain an accurate mg/L COD reading or to generate a correction factor for the precalibrated KHP response. Sample digestion time can be extended up to four hours for samples which are difficult to oxidize.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Chloride Removal Cartridges (CRC)	1	25/pkg	26618-25
Manganese III COD Reagent Vials	1	25/pkg	26234-25
Sulfuric Acid, concentrated, ACS	1 mL	2.5 L	979-09
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Adapter, COD, DR/2010	1	each	44799-00
Blender, Osterizer, 120 Vac, 14-speed	1	each	26747-00
Blender Container, 50-250 mL	1	2/pkg	26748-00
Cap, with inert Teflon liner, for mixing bottle	varies	each	24018-12
Chloride Removal Cartridge	1	25/pkg	26618-25
COD Reactor, 120 V	1	each	45600-00
COD Reactor, 240 V	1	each	45600-02
Forceps, extra fine point	1	each	26696-00
Mixing Bottle, glass, for sample + acid	1	each	24277-00
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette®	2	50/pkg	21997-96
Pipet Tips, for 19700-10 TenSette®	2	250/pkg	21997-25
Pipet, TenSette®, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette®	2	50/pkg	21856-96
Pipet Tips, for 19700-01 TenSette®	2	1000/pkg	21856-28
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack, stainless steel	1	each	18641-00
Vacuum Pretreatment Device (VPD)	1	each	49000-00
Vacuum Pump, 115 V	1	each	14697-00
Vacuum Pump, 230 V	1	each	14697-02

OPTIONAL REAGENTS AND APPARATUS

COD Standard Solution, 800 mg/L COD	200 mL		26726-29
Dispenser, for sulfuric acid		each	25631-37
Potassium Acid Phthalate, ACS	500 g		315-34
Water Quality Test Strips for Low Range Chloride	40/pkg		27449-40

OXYGEN, DISSOLVED*

Azide Modification of Winkler Method With Digital Titrator

Using a 300-mL BOD Bottle — Method 8215

1. Collect a water sample in a clean 300-mL BOD Bottle.

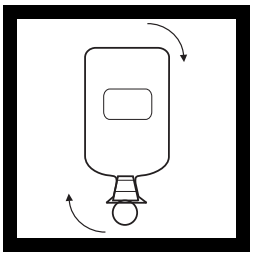
Note: *Allow the sample to overflow the bottle for 2-3 minutes to ensure air bubbles are not trapped.*

Note: *If samples cannot be analyzed immediately, see Sampling and Storage following these steps.*

2. Add the contents of one Manganous Sulfate Powder Pillow and one Alkaline Iodide-Azide Reagent Powder Pillow.

3. Immediately insert the stopper so air is not trapped in the bottle. Invert several times to mix.

Note: *A flocculent precipitate will form. It will be orange-brown if oxygen is present or white if oxygen is absent. The floc settles slowly in salt water and normally requires 5 additional minutes before proceeding to Step 5.*



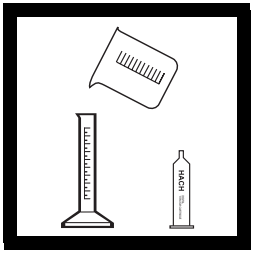
4. Wait until the floc in the solution has settled. Again invert the bottle several times and wait until the floc has settled.

Note: *Waiting until floc has settled twice assures complete reaction of the sample and reagents.*

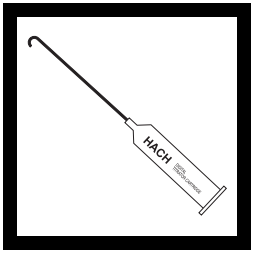
* For reporting purposes, use the USEPA-approved buret titration, available on request (method 8229).

5. Remove the stopper and add the contents of one Sulfamic Acid Powder Pillow. Replace the stopper without trapping air in the bottle and invert the sample several times to mix.

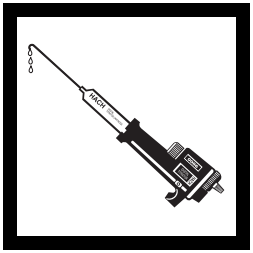
Note: *The floc will dissolve and leave a yellow color if oxygen is present.*



6. Select a sample volume and Sodium Thiosulfate Titration Cartridge corresponding to the expected dissolved oxygen (D.O.) concentration from *Table 1*.

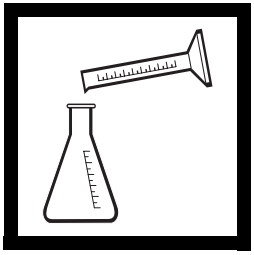


7. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.

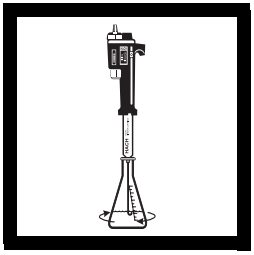


8. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

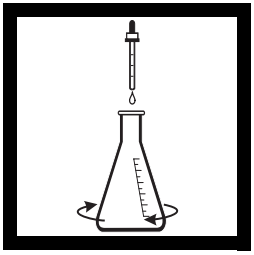
Note: *For added convenience use the TitraStir stirring apparatus.*



9. Use a graduated cylinder to measure the sample volume from *Table 1*. Transfer the sample into a 250-mL Erlenmeyer flask.

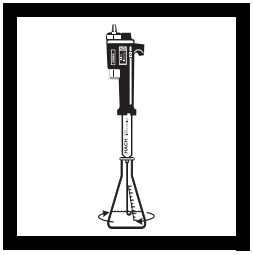


10. Place the delivery tube tip into the solution and swirl the flask while titrating with sodium thiosulfate to a pale yellow color.



11. Add two 1-mL droppers of Starch Indicator Solution and swirl to mix.

Note: *A dark blue color will develop.*



12. Continue the titration to a colorless end point. Record the number of digits required.

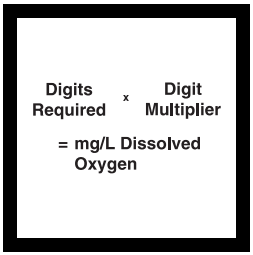


Table 1

Range mg/L	Sample Volume (mL)	Titration Cartridge (N)	Cat. No.	Digit Multiplier
1-5	200	0.200	22675-01	0.01
2-10	100	0.200	22675-01	0.02
>10	200	2.000	14401-01	0.1

13. Calculate:

Digits Required x Digit Multiplier = mg/L Dissolved Oxygen

Using a 60-mL BOD Bottle

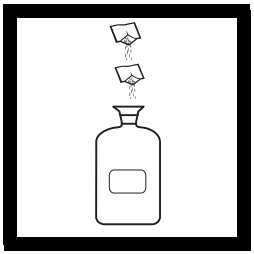
Method 8332

1. Collect a water sample in a clean 60-mL glass-stoppered BOD Bottle.

Note: *Allow the sample to overflow the bottle for 2-3 minutes to ensure air bubbles are not trapped.*

Note: *If samples cannot be analyzed immediately, see Sampling and Storage following these steps.*

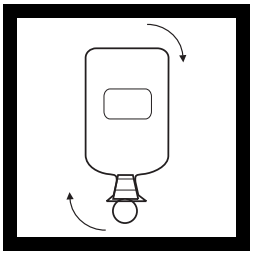
Note: *Follow this procedure when using the 60-mL glass-stoppered BOD Bottle supplied with DREL Portable Laboratories.*



2. Add the contents of one Dissolved Oxygen 1 Reagent Powder Pillow and one Dissolved Oxygen 2 Reagent Powder Pillow

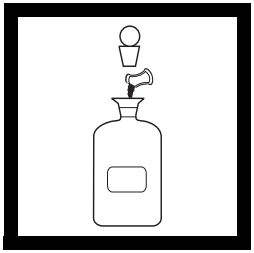
3. Immediately insert the stopper so air is not trapped in the bottle. Invert several times to mix.

Note: *A flocculent precipitate will form. It will be orange-brown if oxygen is present or white if oxygen is absent. The floc settles slowly in salt water and normally requires 5 additional minutes before proceeding to Step 5.*



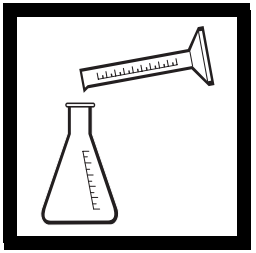
4. Wait until the floc in the solution has settled and the top half of the solution is clear. Again invert the bottle several times and wait until the floc has settled.

Note: *Results are not affected if the floc does not settle or if some of the reagent powder does not dissolve.*

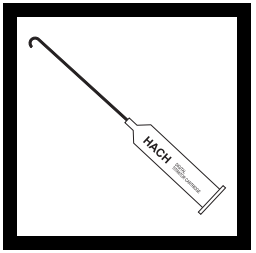


5. Remove the stopper and add the contents of one Dissolved Oxygen 3 Powder Pillow. Replace the stopper without trapping air in the bottle. Invert several times to mix.

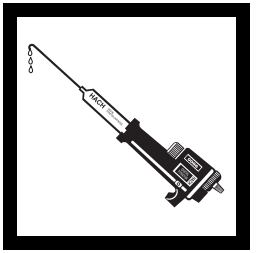
Note: *The floc will dissolve and leave a yellow color if oxygen is present.*



6. Accurately measure 20 mL of the prepared sample and transfer it to a 250-mL Erlenmeyer flask.

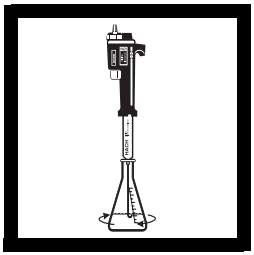


7. Attach a clean straight-stem delivery tube to a 0.2000 N Sodium Thiosulfate Titration Cartridge. Twist the cartridge onto the titrator body.

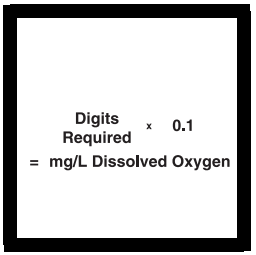


8. Flush the delivery tube by turning the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.

Note: *For added convenience use the TitraStir stirring apparatus.*



9. Titrate the prepared solution with 0.2000 N Sodium Thiosulfate until the sample changes from yellow to colorless. Record the number of digits used.



10. Calculate:

Digits required x 0.1 = mg/L Dissolved Oxygen

Sampling and Storage

Sampling and sample handling are important in obtaining meaningful results. The dissolved oxygen content of the sample changes with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time, and other factors. A single dissolved oxygen test rarely reflects the over-all condition of a body of water. Several samples taken at different times, locations and depths are recommended for most reliable results.

Collect samples in clean BOD Bottles (see step 1). If storage is necessary, run *steps 1–4* of the procedure and store in the dark at 10-20 °C. Seal the bottle with water by pouring a small volume of water into the flared lip area of a stopper bottle. Snap a BOD Bottle Cap over the flared lip. Samples preserved like this can be held 4-8 hours. Begin with step 5 when analyzing.

Accuracy Check

An Iodate-Iodide Standard Solution which is equivalent to 10-mg/L dissolved oxygen is available for checking the strength of the sodium thiosulfate titrant. For the 300-mL procedure, begin at step 5, adding the Sulfamic Acid Powder Pillow. For the 60-mg/L procedure, begin the analysis at Step 5, adding the Dissolved Oxygen 3 Powder Pillow. The titration should take 10 mL. If more than 10.5 mL is required to reach the end point, replace the Sodium Thiosulfate Solution.

Interferences

Nitrite interference is eliminated by the azide in the reagents. Other reducing or oxidizing substances may interfere. If these are present, use an alternate method, such as the *High Range Dissolved Oxygen Method* (colorimetric) or a dissolved oxygen electrode.

Summary of Method

Samples are treated with manganous sulfate and alkaline iodide-azide reagent to form an orange-brown precipitate. Upon acidification of the sample, this floc reacts with iodide to produce free iodine as triiodide in proportion to the oxygen concentration. The iodine is titrated with sodium thiosulfate to a colorless end point.

REQUIRED REAGENTS for 300-mL BOD BOTTLE

(Varies with sample characteristics)

Dissolved Oxygen Reagent Set (about 50 tests) 22722-00
Includes: (2) Cat. No.1072-68, (2) Cat. No.1071-68, (1)Cat. No. 22675-01,
(1) Cat. No. 349-32, (2) Cat. No. 20762-68

Description	Quantity Required Per Test	Unit	Cat. No.
Alkaline Iodide-Azide Powder Pillows	1	50/pkg	1072-66
Manganous Sulfate Powder Pillows	1	50/pkg	1071-66
Sodium Thiosulfate Titration Cartridge, 0.2000 N	1	each	22675-01
Sodium Thiosulfate Titration Cartridge. 2.00 N	1	each	14401-01
Starch Indicator Solution	2 mL	100 mL MDB*	349-32
Sulfamic Acid Powder Pillows	1	50/pkg	20762-66

REQUIRED APPARATUS for 300-mL BOD BOTTLE

Bottle, with stopper, BOD, 300-mL	1	each	621-00
Clippers, for opening pillows	1	each	968-00
Cylinder, graduated, 250-mL	1	each	508-46
Digital Titrator	1	each	16900-01
Flask, Erlenmeyer, 250-mL	1	each	505-46

REQUIRED REAGENTS for 60-mL BOD BOTTLE

Dissolved Oxygen 1 Reagent Powder Pillows	1	100/pkg	981-99
Dissolved Oxygen 2 Reagent Powder Pillows	1	100/pkg	982-99
Dissolved Oxygen 3 Reagent Powder Pillows	1	25/pkg	987-68
Sodium Thiosulfate Titration Cartridge, 0.2000 N	1	each	22675-01

REQUIRED APPARATUS for 60-mL BOD BOTTLE

Bottle, with stopper, BOD, 60-mL	1	each	1909-02
Clippers, for opening pillows	1	each	968-00
Cylinder, graduated, 50-mL	1	each	508-41
Digital Titrator	1	each	16900-01
Flask, Erlenmeyer, 125 mL	1	each	505-43

OPTIONAL REAGENTS

Iodate-Iodide Standard Solution, 10 mg/L as DO ..... 500 mL* 401-49

OPTIONAL APPARATUS

Description	Unit	Cat. No
Cap, BOD Bottle, plastic	6/pkg	2419-06
Clamp Holder	each	326-00
Clamp, 2-prong, extension, 38 mm	each	21145-00
Delivery Tubes, with 180° hook	5/pkg	17205-00
Delivery Tubes, 90° with hook	5/pkg	41578-00
Sewage Sampler, Lab-Line	each	427-00
Support ring stand	each	563-00
TitraStir Mixer/Stand Assembly, 115 Vac	each	19400-00
TitraStir Mixer/Stand Assembly, 230 Vac	each	19400-10

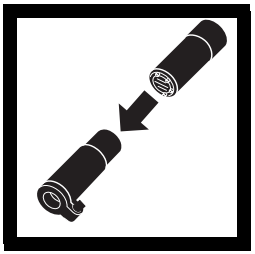
* Contact Hach for larger sizes.

OXYGEN, DISSOLVED

Polarographic Electrode Method (using the Hach *sension*™*6* DO Meter)
Range: 0–20 mg/L O_2

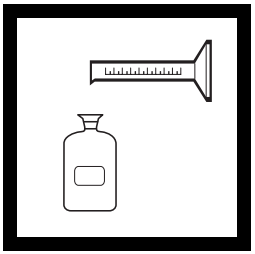
1. Prepare the probe and connect it to the meter.

Note: *See the instrument manual for complete instructions on preparing the probe and using the meter.*



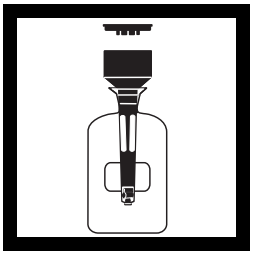
2. Calibrate the probe according to the directions in the meter manual.

Note: *Use the Hach BOD Accessory Kit with a magnetic stirrer and a standard 300-mL BOD bottle.*

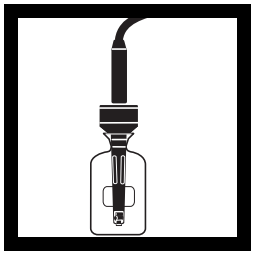


3. Fill a standard 300-mL BOD bottle with sample.

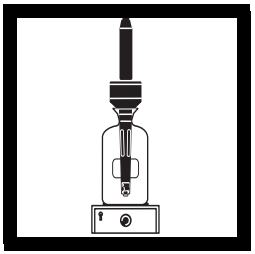
Note: *If measuring a sample stream, lower the probe into the sample. Wait for a stable reading, then record the result.*



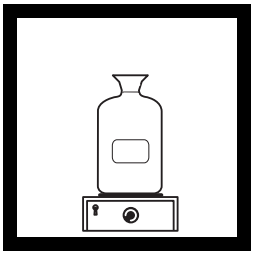
4. Insert the overflow funnel into the BOD bottle.



5. Insert the probe into the funnel/bottle.



6. Place the BOD bottle on a magnetic stirrer so that the probe is over the center of the stir plate.



7. Start the magnetic stirrer and increase the speed until the rotor loses it cycle. Then decrease the speed until the rotor starts spinning. Mark this point on the speed scale on the stirrer. This is the optimum working point.

Note: *Insufficient stirring. will cause low results.*

8. Wait for a stable reading, then record or store the result (i.e, 5.75 mg/L)

Note: *Press* **CONC %** *to change the units from mg/L to % saturation.*

SUMMARY OF METHOD

The dissolved oxygen meter is supplied with a Clark-type polarographic electrode equipped with a temperature compensating thermistor. The sensor is a gold cathode and a silver anode surrounded by a potassium chloride electrolyte.

A thin oxygen permeable Teflon®* membrane stretched over the sensor isolates the sensor from the environment. When a polarizing current is applied across the probe, it reduces oxygen at the cathode, causing a current to flow. The current produced is proportional to the dissolved oxygen in the sample.

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
*sens**ion**™6* Dissolved Oxygen Meter, portable	1	each	51850-00

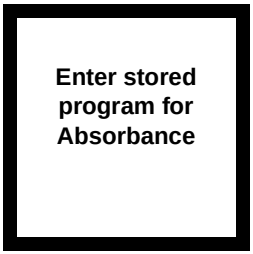
OPTIONAL APPARATUS

Batteries, AA	4/pkg	19380-04
BOD Accessory Kit	each	50182-00
Bottle, BOD, 300 mL, w/ stopper	each	621-00
D.O. Membranes	2/pkg	51973-00
D.O. Filling Solution	59 mL	27591-26
D.O. Probe, 1 meter cable	each	51970-00
D.O. Probe, 3 meter cable	each	51970-03
D.O. Probe, 15 meter cable	each	51970-15
D.O. Service Kit (includes membranes, filling solution, polishing discs, and sponges for LIS chamber)	each	51968-00
Docking Station, for meter, 115 V, North American plug	each	51875-01
Docking Station, for meter, 230 V, Continental European plug	each	51875-02
Electrode Stand with Electromagnetic stirrer, 115 V	each	45300-01
Electrode Stand with Electromagnetic stirrer, 230 V	each	45300-02
Low Ionic Strength Chamber	each	51899-00

* Teflon is a registered trademark of I.E. DuPont.

PCB IN SOIL

Immunoassay Method
Range: 1 or 10 ppm threshold

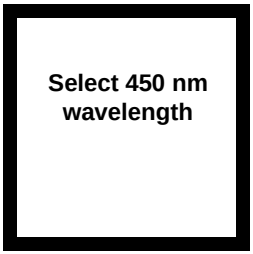


1. Enter the stored program for absorbance.

For DR/2010, press:
0 ENTER

For DR/4000, press the soft key under ***SINGLE*** λ**,** then press the soft key under ***GO TO*** λ.

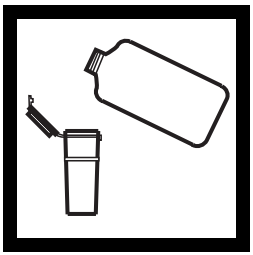
For the DR/800s, press **PRGM 42 ENTER**.



2. Select a wavelength of 450 nm (the DR/800s will automatically select 420 nm).

Note: *The Pour-Thru Cell cannot be used.*

Phase 1 Soil Extraction

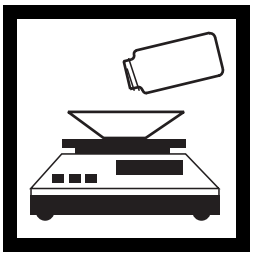


1. Fill the extraction vial to the 0.75-oz line with Soil Extractant Solution.

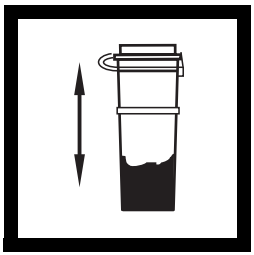
Note: *This is equivalent to adding 20 mL of Soil Extractant Solution.*

2. Place a plastic weighing boat on an analytical balance. Tare the balance.

Note: *Use either the portable AccuLab Pocket Pro or a laboratory balance (see Optional Equipment and Supplies).*

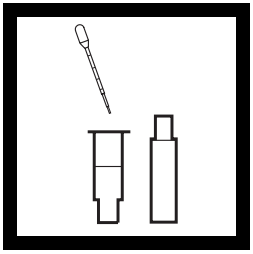


3. Weigh out 10 ±0.1 g of soil in the plastic weighing boat. Carefully pour the soil from the weighing boat into the extraction vial.



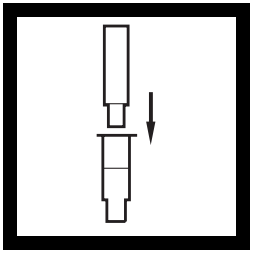
4. Cap the extraction vial tightly and shake vigorously for one minute.

5. Allow to settle for 1 minute. Gently open the extraction vial.



6. Using the disposable bulb pipet, withdraw 1.0–1.5 mL from the liquid (top) layer in the extraction vial. Transfer this aliquot into the filtration barrel (the bottom part of the filtering assembly; the plunger is inserted into it).

Note: *Do not transfer more than 1.5 mL into the barrel. The pipet is marked in 0.25-mL increments.*

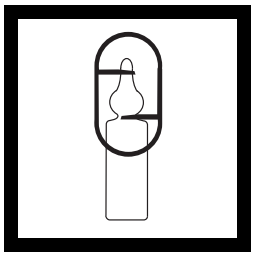


7. Insert the filtration plunger into the filtration barrel. Press firmly on the plunger until at least 0.5 mL of filtered sample is collected in the center of the plunger.

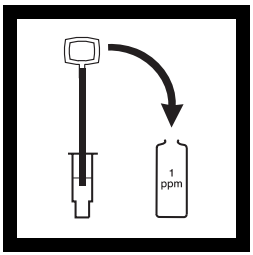
Note: *The liquid will be forced up through the filter. The liquid in the plunger is the filtered extract.*

Note: *It may be necessary to place the filtration assembly on a table and press down on the plunger*

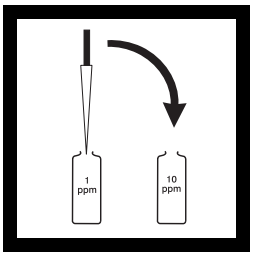
Phase 2 Preparing Sample and Standards



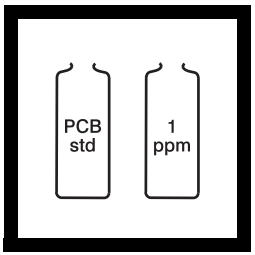
1. To prepare a 1-ppm threshold dilution, snap open a 1-ppm Dilution Ampule. Label the Dilution Ampule with appropriate information.



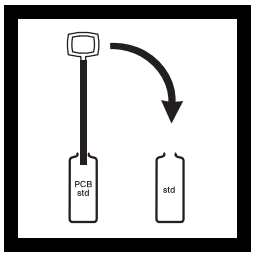
2. Using the WireTrol® pipet, withdraw 100 µL (0.1 mL) of sample extract from the filtration plunger and add it to the 1-ppm Dilution Ampule. Swirl to mix. Discard the capillary tube.



3. To prepare a 10-ppm threshold, snap open a 10-ppm Dilution Ampule. Label the Dilution Ampule. Using a TenSette Pipet, withdraw 1.0 mL from the 1-ppm Dilution Ampule (*step 2*), and add it to the 10-ppm Dilution Ampule. Swirl to mix.



4. To prepare the standard, snap open a PCB Standard Ampule. Snap open a 1-ppm Dilution Ampule. Label the Dilution Ampule as "Standard."

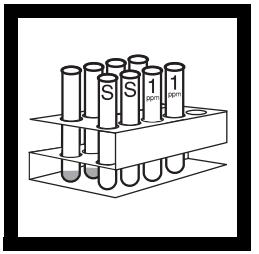


5. Using the WireTrol pipet withdraw 100 µl (0.1 mL) of the standard and add it to the 1-ppm Dilution Ampule. Swirl to mix thoroughly.

Note: *Dispense standard and sample below the level of the solution in the Dilution Ampules.*

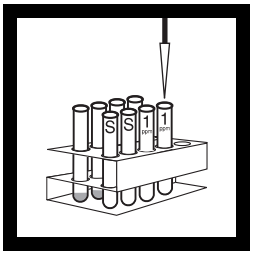
Note: *Use the standard dilution prepared above for both 1-ppm and 10-ppm thresholds. Do not further dilute the standard.*

Phase 3 Immunoassay



1. Label two PCB Antibody Tubes for each dilution ampule. Label two PCB Enzyme Conjugate tubes for each dilution ampule.

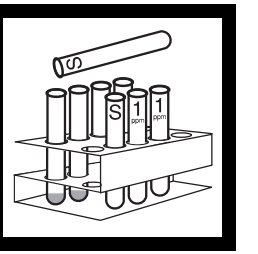
Note: *The PCB Conjugate and PCB Antibody tubes are matched lots. Mixing with other lots will cause erroneous results.*



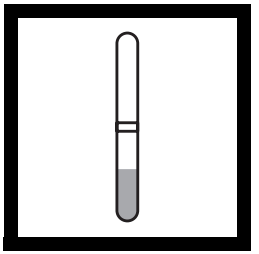
2. Use a TenSette Pipet to add a 1.0-mL aliquot from each dilution ampule prepared (1-ppm or 10-ppm) to the bottom of each appropriately labeled PCB Antibody Tube. Do this for each sample and standard. Use a new pipet tip for each solution.

Note: *Do not touch the inside walls of the tubes.*

3. Begin a 10-minute reaction period.



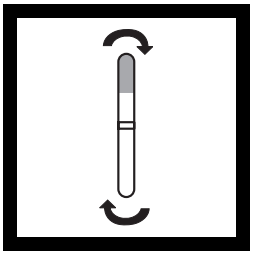
4. At the end of the 10-minute reaction period, decant the solution from the Antibody Tubes into the respective Enzyme Conjugate Tubes.



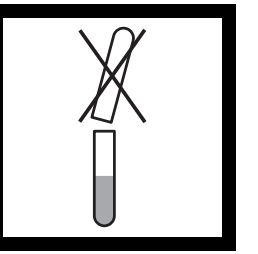
5. Invert and place the Antibody Tubes over the Enzyme Conjugate Tubes until they fit tightly onto the Enzyme Conjugate Tubes.

6. Begin a five-minute reaction period.

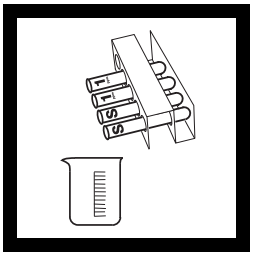
Note: *Immediately proceed with the next step.*



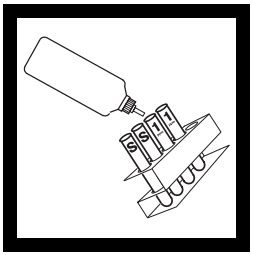
7. Immediately invert the solution repeatedly until the Antibody Tube has been filled four times and the Enzyme Conjugate has been dissolved. After the last inversion make sure that all of the solution is in the Antibody Tube and that it is upright.



8. Place the Antibody Tube in the rack and remove the Enzyme Conjugate Tube from the mouth of the Antibody Tube. Discard the used Enzyme Conjugate Tube.



9. After the five-minute period, discard the contents of the PCB Antibody Tubes into an appropriate waste container.



10. Wash each tube thoroughly and forcefully 4 times with Wash Solution. Empty the tubes into an appropriate waste container. Shake well to ensure most of the Wash Solution drains after each wash.

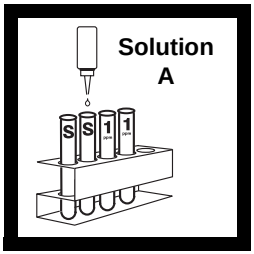
Note: *Wash Solution is a harmless detergent.*

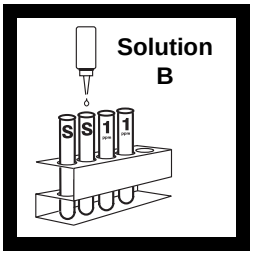
Continue to Phase 4

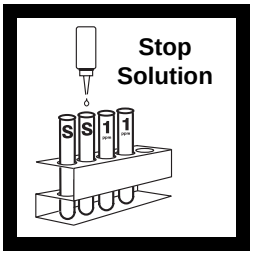
11. Continue to the next phase immediately.

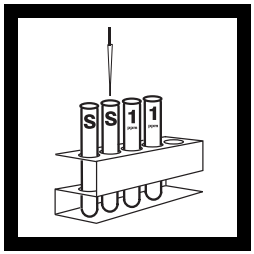
Note: *Ensure most of the Wash Solution is drained from the tubes by turning the tubes upside down and gently tapping them on a paper towel to drain. Some foam may be left from the Wash Solution; this will not affect results.*

Phase 4 Color Development
Check reagent labels carefully.
Reagents must be added in proper order!









1. Add 5 drops of **Solution A** to each tube. Replace the bottle cap.

Note: *Hold all reagent bottles vertically for accurate delivery or erroneous results may occur.*

2. Begin a 2.5-minute period and immediately add 5 drops of **Solution B** to each tube. Swirl to mix. Replace the bottle cap.

Note: *Add drops to the tubes in the same order to ensure proper timing (i.e., left to right) Solution will turn blue in some or all of the tubes.*

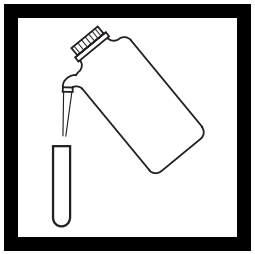
3. Let each tube react for exactly 2.5 minutes. Then add 5 drops of **Immunoassay Stop Solution** to each tube. Replace the bottle cap.

Note: *Blue solutions will turn yellow when Stop Solution is added. PCB concentration is inversely proportional to color development; a lighter color indicates higher levels of PCB.*

4. Using the TenSette Pipet and a new tip, add 0.5 mL of deionized water to each tube.

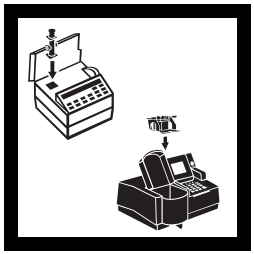
Swirl to mix.

Phase 5 Measuring the Color



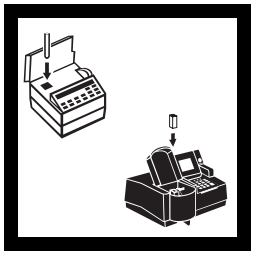
1. Fill a Zeroing Tube with deionized water (the blank). Wipe the outside of all the tubes with a tissue to remove smudges and fingerprints.

Note: *For DR/4000s, fill a 1-cm sample cell; handle by the frosted sides.*

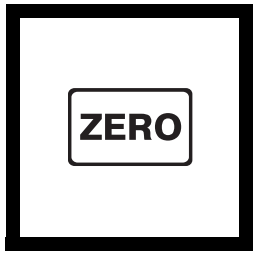


2. Insert the Immuno-assay adapter into the sample cell compartment.

Note: *For DR/4000s, use the Microcell Adapter.*



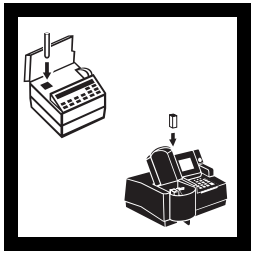
3. Place the blank in the cell holder. Place the cover on the adapter.



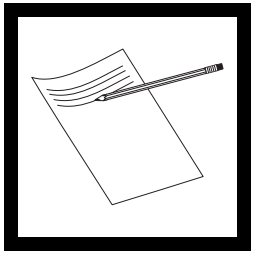
4. Press: **ZERO**

The display will show:

0.000 ABS



5. Insert Standard #1 Antibody Tube into the cell holder. Place the cover on the adapter.

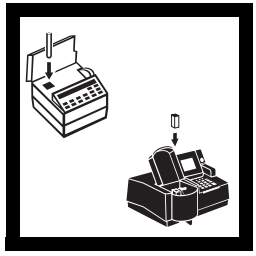


6. Record the absorbance reading.

Repeat Steps 5 and 6 for Standard #2

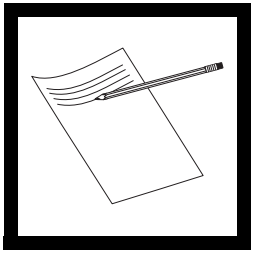
7. Repeat steps 5 and 6 for the Standard #2 Antibody Tube.

Note: *If standard 1 and Standard 2 are more than 0.350 absorbance units apart, repeat the test beginning at Phase 2, Standard Preparation.*



8. Insert the Sample #1 Antibody Tube into the cell holder. Place the cover on the adapter.

Note: *PCB concentration is inversely proportional to the color intensity (or absorbance value). More color means less PCB in the sample.*



9. Record the absorbance reading.

Repeat Steps 8 and 9 for Standard #2

10. Repeat steps *8* and *9* for the Sample #2 Antibody Tube. See *Table 1* below to interpret results.

Interpreting the Results

Use Table 1 to interpret the results.

Table 1

If sample absorbance is. . .	1 ppm Threshold	10 ppm Threshold
. . . less than the highest standard absorbance	Sample PCB is greater than 1 ppm	Sample PCB is greater than 10 ppm
. . . greater than the highest standard absorbance	Sample PCB is less than 1 ppm	Sample PCB is less than 10 ppm

Sensitivity

Compound		Concentration Required to Result in Positive Test at 1-ppm Threshold
PCBs		
	Arochlor 1260	0.4
	Arochlor 1254	0.4
	Arochlor 1248	1
	Arochlor 1242	2
	Arochlor 1016	4
	Arochlor 1232	4
Other Halogenated Compounds		
	2,4,6-trichloro-p-terphenyl	>10,000
	Halowax 1013	10,000
	Halowax 1051	1,000
	o,p-DDT	>10,000
	2,4-D	10,000
	Silvex	1,000
	bifenox	1,000
	tetradifon	100
	Dicofop, methyl	1,000
	dichlorofenthion	10,000
	trichloroethylene	>10,000
	1,2,4-trichlorobenzene	10,000
	2,4-dichloro-1-naphthol	50
	2,4-dichlorophenyl benzene sulfonate	1,000
	1-chloronaphthalene	>10,000
	pentachlorobenzene	>10,000
	hexachlorobenzene	>10,000
	2,5-dichloroaniline	>10,000
Miscellaneous Compounds		
	toluene	>10,000
	Naphthalene	>10,000
	DIALA(R) Oil AX	>10,000
	R-Temp fluid	>10,000
	Envirotemp 200 fluid	>10,000
	diesel fuel	>10,000
	gasoline	>10,000

Storing and Handling Reagents

- Wear protective gloves and eye wear.
- Store reagents at room temperature and out of direct sunlight (less than 80 °F or 27 °C).
- Keep aluminized pouch that contains antibody-coated tubes sealed when not in use.
- If Stop Solution or liquid from the extraction jar comes in contact with eyes, wash thoroughly with cold water and seek immediate medical help.
- Operational temperature of the reagents is 40–90 °F (5–32 °C).

Accuracy Check

To confirm results, use the standard method approved by the USEPA, Method 8015. This method is equivalent to SW-846 Method 4020 (USEPA letter, 1994).

Summary of Method

Samples, standard, and color-development reagents are added to test tubes coated with an antibody specific for PCB. The sample PCB concentration is determined by comparing the developed color intensity to that of a PCB standard. The PCB concentration is inversely proportional to the color development; a lighter color indicates a higher PCB concentration.

Pollution Prevention and Waste Management

The soil extractant (methanol) is an ignitable (D001) waste regulated by the Federal RCRA. Collect this material with laboratory solvents for disposal. If the soil samples being analyzed are contaminated with hazardous waste, the samples and resulting test waste may also need to be disposed of in accordance with RCRA.

REQUIRED REAGENTS AND STANDARDS

	Cat. No.
PCB Reagent Set (5 tests)	25859-00
Includes all consumable reagents and apparatus used in the test except PCB Standard Ampules	
PCB Standard Ampules, U.S.A. orders, 350 µg/L, 1 mL	25851-05
(required reagent, must be ordered separately from the PCB Reagent Set. Not available for shipment outside the U.S.)	
PCB Standard Ampules, International orders, 350 µg/L, 1 mL	26663-05
(required reagent, must be ordered separately from the PCB Reagent Set)	

REQUIRED EQUIPMENT AND SUPPLIES

Description	Quantity Required Per Test	Unit	Cat. No.
Adapter, Immunoassay, DR/2010	1	each	46820-00
Adapter, Microcell, DR/4000	1	each	48588-00
Adapter, Immunoassay, DR/800	1	each	48467-00
Clippers, large, for opening powder pillows	1	each	968-00
Gloves, poly, medium (large 25504-03)	1 pair	100/box	25504-02
Goggles, safety, vented	1	each	25507-00
Microcell, 1-cm, for DR/4000, disposable	4	100/pkg	26295-00
Pen, laboratory, permanent, black	1	each	20920-00
Pipet, TenSette®, 0.1-1.0 mL	1	each	19700-01
Pipet Tips, TenSette®, for 19700-01	varies	50/pkg	21856-96
Timer	1	each	23480-00
WireTrol® Pipet, 50/100 µL, w/ 250 capillary tubes	varies	each	25689-05
Zeroing Tube	1	each	26228-00
Rack, grip style	1	each	25873-00

OPTIONAL REAGENTS AND STANDARDS

Water, deionized	4 liters		272-56
Water, deionized	100 mL		272-42

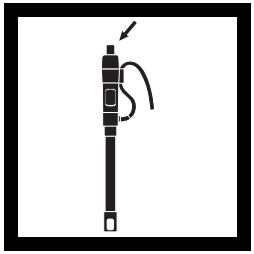
OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker		each	21968-00
Balance, AccuLab		each	25568-00
Balance, Laboratory, Model SL 500		each	26105-00

pH

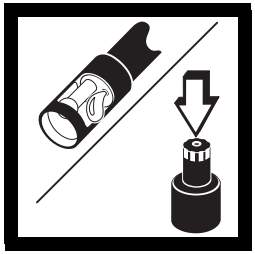
Platinum Series Combination Electrode Method (with *sens*ion™ pH meters)

Sample pH Measurement (calibration required)

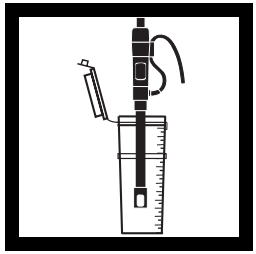


1. Press the Dispenser Button once (it will click).

Note: *See the two-point calibration on the next page to calibrate the meter.*

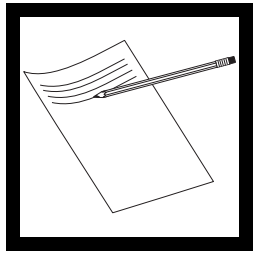


2. Inspect the end of the electrode for the presence of gel. If gel is not visibly oozing from the tubing, press the Dispenser Button again.



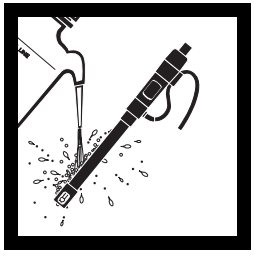
3. Place the electrode in the sample. Be sure the entire sensing end is submerged and that there are no air bubbles under the electrode.

Note: *Directly measure the pH of aqueous solutions. For solids, sludges, and nonaqueous liquids, mix an approximate 10% slurry of the substance and deionized water. Then take measurements on the aqueous portion.*



4. Record the pH value when the display is stable.

Note: *The sens**ion**2**, **3** and **4** can store sample information. See the meter manual for more information.*

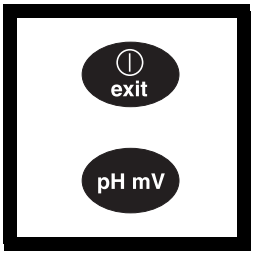


5. Rinse the electrode thoroughly with deionized water and blot dry.

Two-Point Calibration (with Temperature Probe)

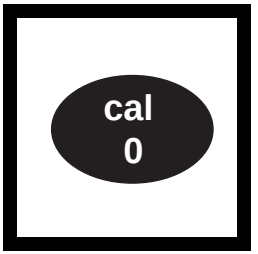
1. Prepare two buffers of 4 and 7 pH or 7 and 10 pH. Turn the meter on. The standards should bracket the sample pH.

Note: *Other buffers may be used for calibration. See the meter manual for details.*



2. Turn the meter on. by pressing the **I/EXIT** key. If necessary, press the **PH/MV** key to put the meter in pH mode.

Note: *Be sure no air bubbles are trapped inside the glass bulb of the electrode and that gel is present at the tubing end.*

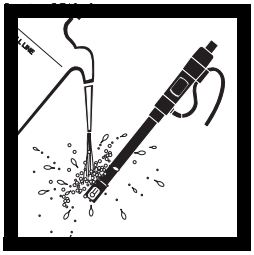


3. Press the **CAL** key. The display will show **Standard 1?**

Note: *Hach buffers are available as powders or solutions. They are color-coded for convenience.*

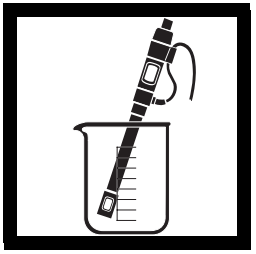
4. Place the electrode into a pH 4 buffer solution. Press **ENTER**. The display will show **Stabilizing...**

Note: *Buffer solutions (pH 4, 7 and 10) may be used in any order.*

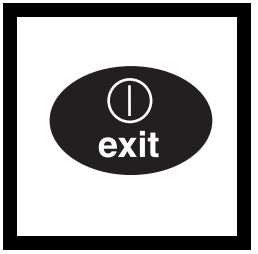


5. When a stable pH is determined, the display will show **Standard 2?**.

6. Remove the electrode from the cup, rinse, and blot dry.

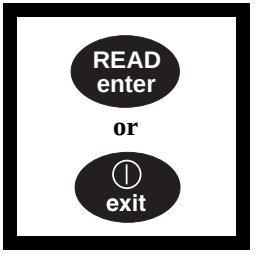


7. Place the electrode in 7.0 (or 10.0) pH buffer.
Press **ENTER**.



8. After the last calibration point has stabilized and the display reads **Standard 3?,** press **EXIT**.

Note: *For a three-point calibration, repeat steps 11-13 with an additional buffer before pressing* **EXIT**.



9. The display will show **Store?**. Press **ENTER** to store the calibration or **EXIT** to leave the calibration mode without storing the value.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Cool to 4 °C (39 °F) and determine pH within six hours. If samples cannot be analyzed within six hours, report the actual holding time with the results.

Interferences

- Acid error is negligible.
- Sodium error, usually present in alkaline solutions, is low, but increases at pH values higher than pH 11.

Platinum Series pH Electrode

Storage

Proper storage, conditioning, and maintenance of pH electrodes is critical. The glass sensing membrane is a dynamic material that needs proper care to function properly. The optimum storage of the electrode is an environment that both hydrates the glass and also creates an osmotically-neutral effect to avoid leaching the pH-sensing properties of the glass. pH Electrode Storage Solutions, or appropriate buffer solution such as pH 7 buffer, create this environment very well, offering chemical components that assist hydration and create enough ionic strength to avoid leaching salts from the glass. Electrode Storage Solutions also maintain an approximately-neutral pH environment.

To properly address the storage and conditioning of electrodes, it is important to consider the sample type to be tested. An electrode that has been reserved in storage solution, buffer, or other strong ionic solution for a long time will take longer to respond in samples of low ionic strength. This slow response is due to a carry-over effect from the buffering components and a purging or reset of the sensing membrane.

Short term storage and long term storage (more than two weeks) require different treatments. See the electrode manual for complete storage instructions.

Normal Cleaning

If the electrode response time slows, the cause may be a fouled reference element or a contaminated glass bulb. Usually, fouling of the reference element is non-reversible; however, sometimes glass bulbs can be rejuvenated. Normal cleaning or reconditioning of the electrode can be performed in the following manner:

Immerse the electrode tip in 0.1 N Hydrochloric Acid followed by immersion in 0.1N Sodium Hydroxide and again in 0.1N Hydrochloric Acid, each for a 2-minute period. Rinse with deionized water and soak in deionized water for at least 15 minutes.

Remove oils and fats by immersing the electrode tip in a detergent solution such as Alconox™. Use a brush or ultrasonic bath if necessary. **Avoid scratching the glass bulb.**

Remove organic films from the glass bulb by using an appropriate solvent, such as methanol or acetone.

Conditioning of the electrode is required after every cleaning, prior to use. If these steps fail to improve electrode response, replace the electrode.

Summary of Method

The Platinum Series Combination pH Electrode responds to the hydrogen ion concentration (activity) by developing an electrical potential at the glass/liquid interface. At a constant temperature, this potential varies linearly with the pH of the solution being measured. The electrode has a free-diffusion junction that eliminates clogging problems and has been proved to provide fast, accurate results.

REQUIRED REAGENTS AND APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
Buffer Powder Pillows, pH 4 and 7, (10 of each)	1	20/pkg	22992-64
Beaker, Griffin, 100 mL	1	each	22994-42
Clippers, for opening powder pillows	1	each	968-00
KCl Reference Electrolyte Gel Cartridge	1	3/pkg	25469-02
pH Meter, portable, *sension™1*, w/ electrode	1	each	51700-10
Platinum Series Combination pH Electrode w/Temp	1	each	51910-00

OPTIONAL REAGENTS

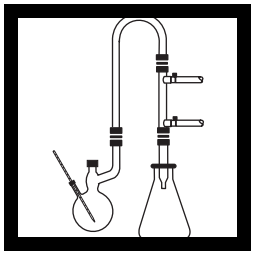
Buffer Powder Pillows, pH 4 (red)	50/pkg	22269-66
Buffer Powder Pillows, pH 7 (yellow)	50/pkg	22270-66
Buffer Powder Pillows, pH 9	50/pkg	14107-66
Buffer Powder Pillows, pH 10 (blue)	50/pkg	22271-66
Buffer Solution, pH 4 (red)	500 mL	22834-49
Buffer Solution, pH 7 (yellow)	500 mL	22835-49
Buffer Solution, pH 10 (blue)	500 mL	22836-49
pH Electrode Storage Solution	500 mL	50301-49
pH Electrode Storage Solution Powder Pillows	20/pkg	26573-64
Water, deionized	4 L	272-56

OPTIONAL APPARATUS

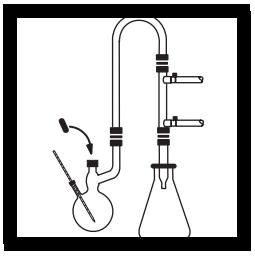
Beaker, poly, 50 mL	each	1080-41
Clippers, for opening powder pillows	each	968-00
Comb. pH Electrode with Temperature (Gel Filled)	each	51935-00
Comb. pH Electrode (Refillable)	each	51910-00
Conductivity Meter, portable, *sension™5*	each	51800-10
Cylinder, mixing, graduated, 50 mL	each	1896-41
Electromagnetic stirrer and electrode holder, (115 V North American)	each	45300-01
Electromagnetic stirrer and electrode holder, (230 V)	each	45300-02
pH/ISE Meter, portable, *sension™2*	each	51725-10
pH Meter, laboratory, *sension™3*	each	51750-10
pH/ISE Meter, laboratory, *sension™4*	each	51775-10
Stir Bar, 22.2 x 4.76 mm ($^{7}/_{8}$ x $^{3}/_{16}$ in.)	each	45315-00
Temperature Probe, 5-pin	each	51980-00
Temperature Probe, 3.5 mm phone-tip	each	51980-11
Temperature Probe, DIN	each	51980-22
Thermometer, armored, -10 to 110 °C	each	1877-01

PHENOLS

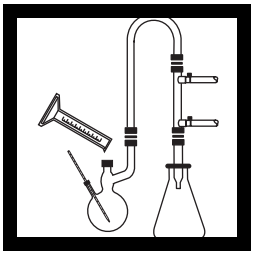
4-Aminoantipyrene Method*
Range: 0–0.200 mg/L
(USEPA accepted for reporting wastewater analysis)**



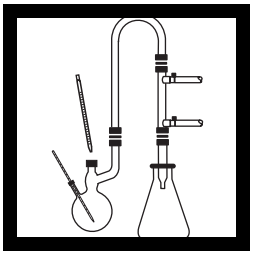
1. Set up the distillation apparatus for the test by assembling the general purpose apparatus as shown in the Hach Distillation Apparatus Manual. Use the 500-mL erlenmeyer flask to collect the distillate. It may be necessary to elevate the flask with a laboratory jack.



2. Place a stir bar into the distillation flask.

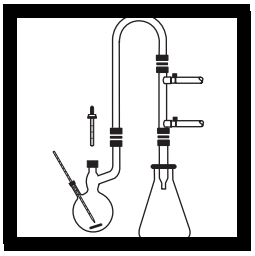


3. Measure 300 mL of sample in a 500-mL graduated cylinder. Pour it into the distillation flask.

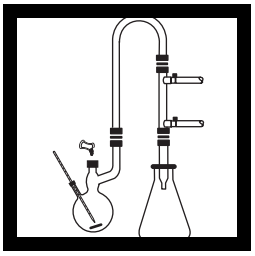


4. Using a serological pipet, add 1 mL of Methyl Orange Indicator Solution to the distillation flask.

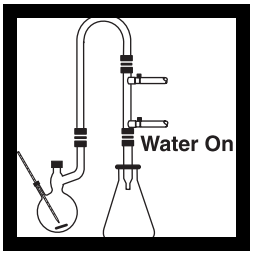
5. Turn on the heater power switch. Set the stir control to 5.



6. Add Phosphoric Acid Solution, 10%, drop-wise, until the indicator changes from yellow to orange.

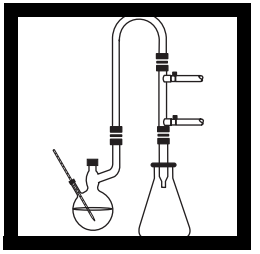


7. Add the contents of one Copper Sulfate Powder Pillow and allow to dissolve (omit this step if copper sulfate was used to preserve the sample).

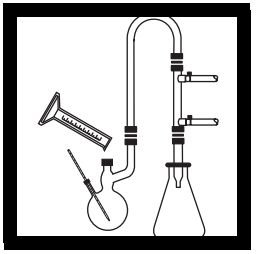


8. Turn on the water and adjust so a constant flow of water is maintained through the condenser. Set the heat control to 10.

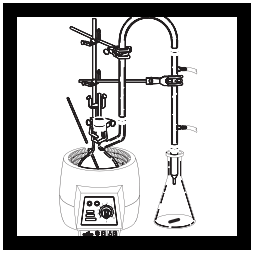
* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Procedure is equivalent to USEPA method 420.1 for wastewater.



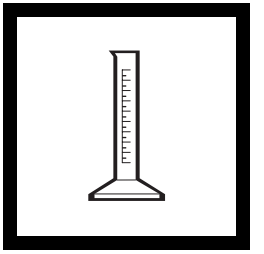
9. Collect 275 mL of distillate in the flask, then turn the still off.



10. Using a graduated cylinder, add 25 mL of deionized water to the distillation flask.

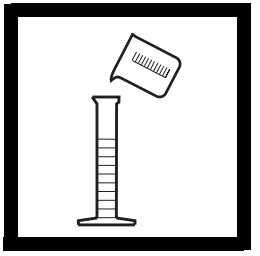


11. Turn the still back on. Heat until another 25 mL of distillate is collected.



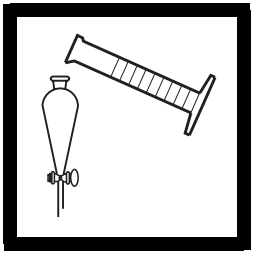
12. Using a clean, dry graduated cylinder, re-measure the distillate to be sure 300 mL has been collected. Proceed to the colorimetric procedure.

Colorimetric Procedure

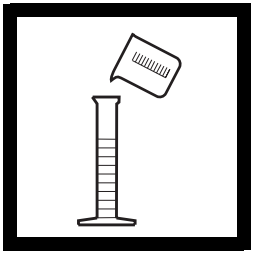


1. Measure 300 mL of deionized water in a 500-mL graduated cylinder.

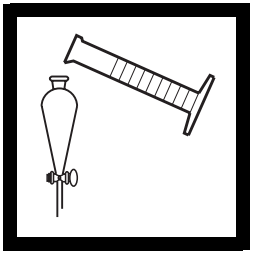
Note: *Analyze samples within four hours to avoid oxidation; see Sampling and Storage following these steps.*



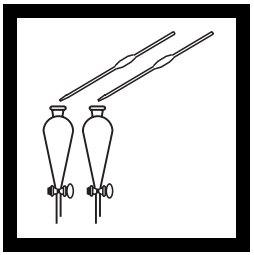
2. Pour the measured deionized water into a 500-mL separatory funnel (the blank).



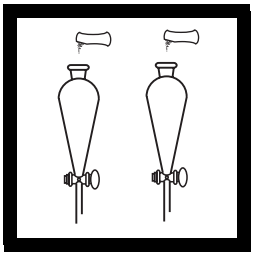
3. Measure 300 mL of sample in a 500-mL graduated cylinder.



4. Pour the measured sample into another 500-mL separatory funnel (the prepared sample).

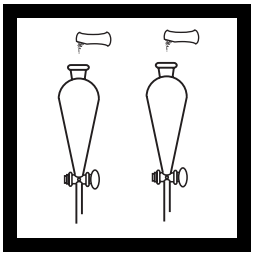


5. Add 5 mL of Hardness 1 Buffer to each separatory funnel. Stopper. Shake to mix.

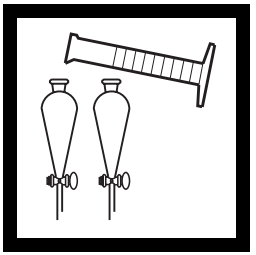


6. Add the contents of one Phenol Reagent Powder Pillow to each separatory funnel. Stopper. Shake to dissolve.

Note: *Spilled reagent will affect test accuracy and is hazardous to skin and other materials.*

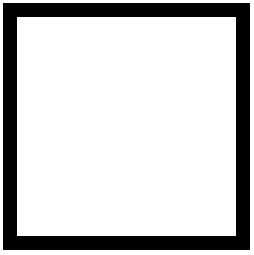


7. Add the contents of one Phenol 2 Reagent Powder Pillow to each separatory funnel. Stopper. Shake to dissolve.

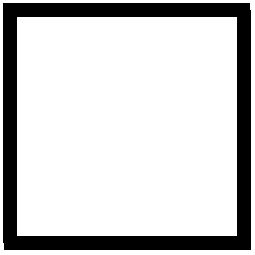


8. Add 30 mL of chloroform to each separatory funnel. Stopper each funnel.

Note: *Use chloroform only with proper ventilation. A fume hood is ideal.*

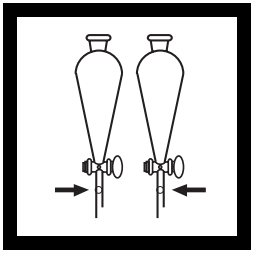


9. Invert each funnel and temporarily vent. Shake each funnel briefly and vent. Then vigorously shake each funnel for a total of 30 seconds.

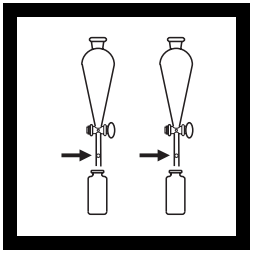


10. Remove the stoppers. Allow both funnels to stand until the chloroform settles to the bottom of the funnel.

Note: *The chloroform will be yellow to amber if phenol is present.*



11. Insert a pea size cotton plug into the delivery tube of each funnel.

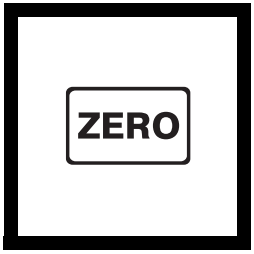


12. Drain the chloroform layer into separate 25-mL sample cells.

Note: *The cotton removes any suspended water or particles from the chloroform.*

Note: *Proceed promptly through the rest of the procedure; chloroform will evaporate, causing high readings.*

Note: *The Pour or Flow-Thru cell can be used.*



13. Place the blank into the cell holder. Close the light shield.

Zero the instrument with the blank, using the settings below.

DR/800s
Program No. NA

DR/2010
Program No. 470
460 nm

DR/4000
Program No.2900
460 nm

14. Place the sample in the cell holder. Read the mg/L phenols.

Sampling and Storage

The most reliable results are obtained when samples are analyzed within four hours after collection. The following storage instructions are necessary only when prompt analysis is impossible. Collect 500 mL of sample in clean glass containers and add the contents of two Copper Sulfate Powder Pillows. Adjust the sample pH to 4 or below with 10% Phosphoric Acid Solution. Store at 4 °C (39 °F) or lower. Analyze within 24 hours.

Accuracy Check

Standard Solution Method
Verify accuracy of the test by performing the analysis using known phenol standard solutions in place of the test sample. For greatest accuracy, standard solutions should be analyzed

periodically to verify test accuracy and when new reagent lots are first used. Prepare standards as follows:

a. Weigh out 1.00 g of phenol, ACS. Transfer to a 1-liter volumetric flask. Dilute to the mark with freshly boiled and cooled deionized water. This is a 1-g/L stock solution.

b. Transfer 1 mL of the 1-g/L stock solution to a 100-mL volumetric flask. Dilute to the mark with deionized water. This is a 10-mg/L working solution.

c. Prepare 0.06, 0.12 and 0.18 mg/L standard solutions by using a pipet to add 3, 6, and 9 mL of the 10-mg/L working solution, respectively, to three separate 500-mL volumetric flasks. Dilute each to the mark with deionized water.

d. Perform the procedure with each of the three standard solutions and verify that the test results are correct.

Interferences

The sample pH must be between 3 and 11.5 for the best results. In the presence of sulfides or suspended matter, the following pretreatment will be necessary:

a. Fill a clean 500-mL graduated cylinder to the 350-mL mark with sample. Pour the sample into a clean 500-mL Erlenmeyer flask.

b. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix.

c. Filter 300 mL of the sample through a folded filter paper. Use this solution in *step 4* of the colorimetric procedure.

Interference can be caused by reducing agents and oxidizing agents such as chlorine.*

* Monitor chlorine using Hach Water Quality Chlorine Test Strips (27450-50).

Summary of Method

The 4-aminoantipyrine method determines all ortho- and meta-substituted phenols or napthols but not para-substituted phenols. These phenols react with 4-aminoantipyrine in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is then extracted from aqueous solution with chloroform and the color is measured at 460 nm.

Sensitivity of the method varies with the type of phenolic compound. Because a water sample may contain various types of phenolic compounds, the results of the test are expressed as the equivalent concentration of phenol. Wastewater and seawater samples may require pretreatment.

REQUIRED REAGENTS

	Cat. No.
Phenols Reagent Set (100 Tests)	22439-00

Includes: (3) 424-49, (2) 14458-17, (2) 1836-99, (2) 872-99

Description	Quantity Required Per Test	Unit	Cat. No.
Buffer Solution, Hardness 1	10 mL	500 mL	424-49
Chloroform, ACS	60 mL	4 L	14458-17
Phenol 2 Reagent Powder Pillow	2 pillows	100/pkg	1836-99
Phenol Reagent Powder Pillow	2 pillows	100/pkg	872-99
Water, deionized	300 mL	4 L	272-56

REQUIRED APPARATUS

Clippers, for opening powder pillows	1	each	968-00
Cotton Balls	2	100/pkg	2572-01
Cylinder, graduated, 50 mL	1	each	508-41
Cylinder, graduated, 500 mL	1	each	508-49
Funnel, separatory, 500 mL	2	each	520-49
Pipet, volumetric, Class A, 5.00 mL	1	each	14515-37
Ring, support, 10 cm (4 in.)	2	each	580-01
Stand, support, 12.5 x 20 cm (5 x 8 in.) base	1	each	563-00

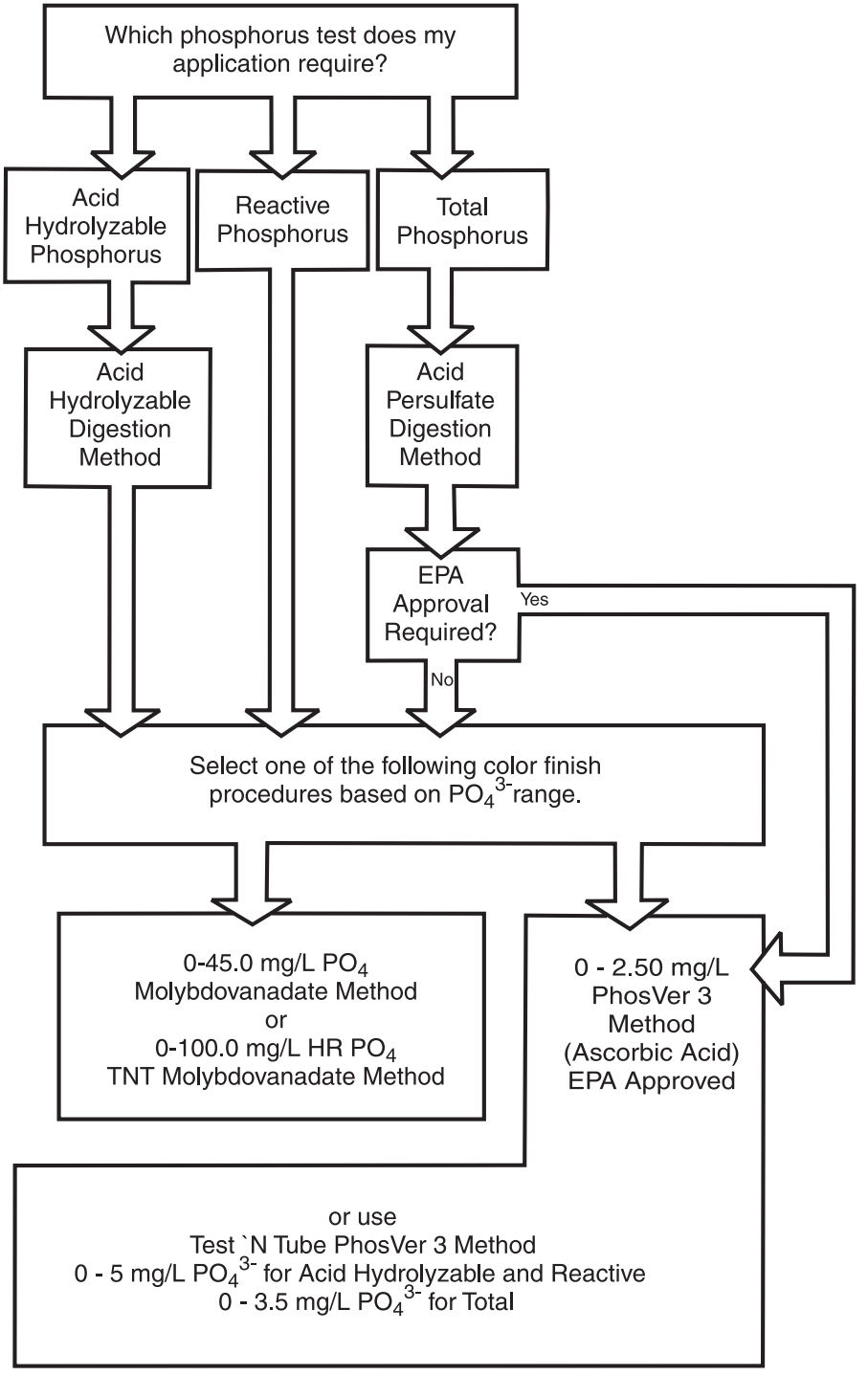
OPTIONAL REAGENTS

Description	Unit	Cat. No.
Copper Sulfate Powder Pillows	50/pkg	14818-66
Methyl Orange Indicator Solution	500 mL	148-49
Phenol, ACS	113 g	758-14
Phosphoric Acid Solution, 10%	100 mL	14769-32
Sulfide Inhibitor Reagent Powder Pillows	100/pkg	2418-99
Water Quality Test Strips, chlorine	50/pkg	27450-50

OPTIONAL APPARATUS

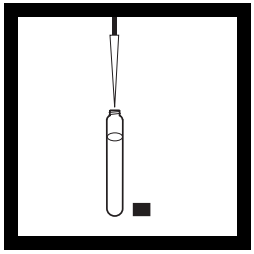
Balance, AccuLab Pocket Pro 150B, handheld, 9V	each	25568-00
Cylinder, 25 mL, graduated	each	508-40
Distillation Apparatus General Purpose Accessories	each	22653-00
Distillation Apparatus Heater, 115 V	each	22744-00
Distillation Apparatus Heater, 230 V	each	22744-02
Filter Paper, 12.5 cm folded	100/pkg	1894-57
Flask, Erlenmeyer, 500 mL	each	505-49
Flask, volumetric, Class A, 100 mL	each	14574-42
Flask, volumetric, Class A, 500 mL	each	14574-49
Flask, volumetric, Class A, 1000 mL	each	14574-53
Funnel, 65 mm poly	each	1083-67
Jack, laboratory	each	22743-00
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipet, serological, 1.0 mL	each	532-35
Pipet, volumetric, Class A, 1.00 mL	each	14515-35
Pipet, volumetric, Class A, 3.00 mL	each	14515-03
Pipet, volumetric, Class A, 6.00 mL	each	14515-06
Pipet, volumetric, Class A, 9.00 mL	each	14515-09
Pipet Filler, safety bulb	each	14651-00

Selecting the Correct Phosphorus Procedure



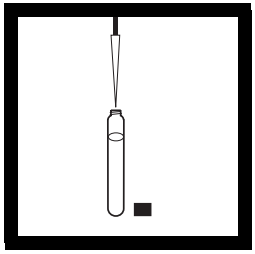
PHOSPHORUS, REACTIVE, High Range

Molybdovanadate Method, Test 'N Tube Procedure*
Range: 0.00 to 100.0 mg/L PO_4^{3}



1. Use a TenSette Pipet to add 5.0 mL of deionized water to a Reactive High Range Phosphorus Test 'N Tube Vial (the blank).

Cap and invert to mix.

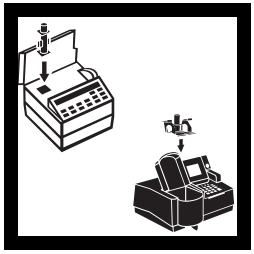


2. Use a TenSette Pipet to add 5.0 mL of sample to a Reactive High Range Phosphorus Test 'N Tube Vial (the sample).

Cap and invert to mix.

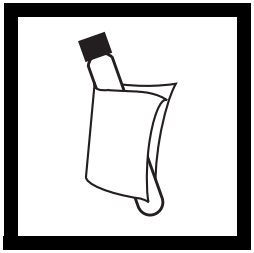
Note: *For non-preserved samples with extreme pH, see INTERFERENCES following these steps.*

3. Begin a two-minute reaction period.



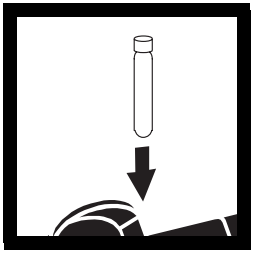
4. During the reaction period, place the COD Vial adapter into the cell holder.

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

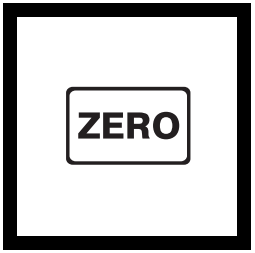


5. Clean the outside of the vial with a towel.

Note: *Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.*



6. Place the sample vial in the adapter. Place the cover on the adapter.

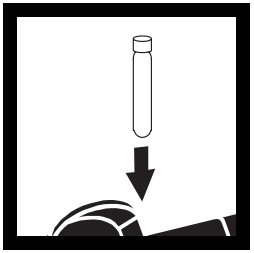


7. Zero the instrument with the vial, using the settings below.

DR/800s
Program No. 86

DR/2010
Program No. 540
420 nm

DR/4000
Program No. 3000
420 nm



8. Place the sample vial into the adapter. Read the mg/L phosphate.

Sampling and Storage

Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

For best results, analyze the samples immediately after collection. If prompt analysis is impossible, preserve the samples for up to 48 hours by filtering immediately and storing at 4 °C. The sample should have a neutral (6–8) pH and be at room temperature before analysis.

Accuracy Check

Note: *Clean glassware with 1:1 hydrochloric acid solution. Rinse again with deionized water. Do not use detergents containing phosphates to clean glassware.*

Standard Additions Method

a. Fill three 10-mL graduated mixing cylinders with 10 mL of sample.

b. Snap the neck off a Phosphate Standard Solution, 500 mg/L as PO_4^{3-} (Cat. No. 14242-10).

c. Use a TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL, respectively, to the three 10-mL aliquots of sample prepared in *step a*. Mix well.

d. Analyze each sample from *step c* as described in the procedure. Use 5.0 mL of the prepared sample for each test. The concentration should increase: 5 mg/L, 10 mg/L, and 15 mg/L PO_4^{3-}, respectively.

e. If these increases do not occur, an interference is likely.

Standard Solution Method

To check accuracy, prepare an 80 mg/L PO_4^{3-} standard by pipetting 8.0 mL of solution from a Voluette Ampule Standard for Phosphate, 500 mg/L as PO_4^{3-}, into an acid-cleaned 50-mL Class A volumetric flask. Fill to the line with deionized water. Substitute this standard for the sample and perform the procedure as described.

Interferences

Large amounts of sample turbidity may cause inconsistent results in the test because the acid present in the reagents may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.

The following may interfere when present in concentrations exceeding these listed below:

Interfering Substance	Interference Level and Treatment
Arsenate	Only interferes if the sample is heated.*
Iron, ferrous	Blue color caused by ferrous iron does not interfere if iron concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Only interferes if the sample is heated.*
Sulfide	Causes a negative interference. Remove interference as follows: **1.** Measure 25 mL of sample into a 50-mL beaker. **2.** Add Bromine Water drop-wise with constant swirling until a permanent yellow color develops. **3.** Add Phenol Solution drop-wise until the yellow color just disappears. Proceed with step 1.
Extreme pH or highly buffered samples	May exceed buffering capacity of the reagents. Samples may require pretreatment. Sample pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause negative interference
Temperature	Cold samples — cause a negative interference. For example, a sample at a temperature of 13 °C (55 °F) has a reaction time of 15 minutes. Hot samples — cause a positive interference. For example, a sample at a temperature of 33 °C (91 °F) has a reaction time of 2 minutes.
The following do not interfere in concentrations up to 1000 mg/L: Pyrophosphate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, salicylate, Al^{3+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Li^{+}, Na^{+}, K^{+}, NH_4^{+}, Cd^{2+}, Mn^{2+}, NO_3^{-}, NO_2^{-}, SO_4^{2-}, SO_3^{2-}, Pb^{2+}, Hg^{+}, Hg^{2+}, Sn^{2+}, Cu^{2+}, Ni^{2+}, Ag^{+}, U^{4+}, Zr^{4+}, AsO_3^{-}, Br^{-}, CO_3^{2-}, ClO_4^{-}, CN^{-}, IO_3^{-}, SiO_4^{4-}.	

* Gentle warming of the sample to reach room temperature will not cause this substance to interfere.

Sample Disposal Information

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Consult the Material Data Safety Data Sheet for information specific to the reagents used.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration.

REQUIRED REAGENTS

Description		Cat. No.
High Range Reactive Phosphorus Test 'N Tube™ Reagent Set	50 vials	27673-45
Includes: (50) Reactive HR Phosphorus Test 'N Tube™ Reagent Vials*, (2) 272-42		

Description	Quantity Required Per Test	Unit	Cat. No.
Reactive HR Phosphorus Test 'N Tube™ Reagent Vials	1	50/pkg	*
Water, deionized	5 mL	100 mL	272-42

REQUIRED APPARATUS

COD Vial Adapter, DR/2010	1	each	44799-00
COD Vial Adapter, DR/4000	1	each	48189-00
COD Vial Adapter, DR/800	1	each	48464-00
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	1	50/pkg	21997-96
Test Tube Rack	1–3	each	18641-00

* These items are not sold separately.

OPTIONAL REAGENTS

Description	Unit	Cat. No.
Bromine Water, 30 g/L	29 mL*	2211-20
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phenol Solution, 30 g/L	29 mL	2112-20
Phosphate Standard Solution, 50 mg/L as PO_4^{3-}	500 mL	171-49
Phosphate Standard Solution, PourRite™ ampule, 500 mg/L as PO_4^{3-}, 2 mL	20/pkg	14242-20
Phosphate Standard Solution, Voluette™ ampule, 500 mg/L as PO_4^{3-}, 10 mL	16/pkg	14242-10

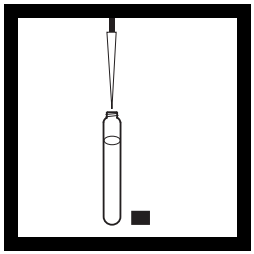
OPTIONAL APPARATUS

Ampule Breaker Kit	each	21968-00
Aspirator, vacuum	each	2131-00
Cylinder, graduated, mixing, 10 mL, 3 required	each	20886-40
Filter Holder, 47 mm, 300 mL, graduated	each	13529-00
Filter, membrane, 47 mm, 0.45 microns	100/pkg	13530-00
Flask, filtering, 500 mL	each	546-49
Flask, volumetric, Class A, 50-mL	each	14574-41
pH Indicator Paper, 1 to 11 pH units	5 rolls/pkg	391-33
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet Tips, for 19700-10 TenSette® Pipet	1000/pkg	21997-28
Pipet, volumetric, Class A, 8.00-mL	each	14515-08
PourRite™ Ampule Breaker	each	24846-00

* Larger sizes available.

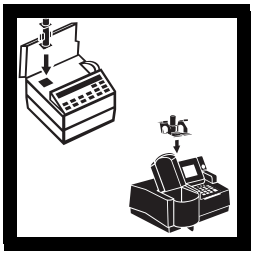
PHOSPHORUS, REACTIVE

PhosVer 3 Test 'N Tube Procedure*
Range: 0.00–5.00 mg/L PO_4^{3-}

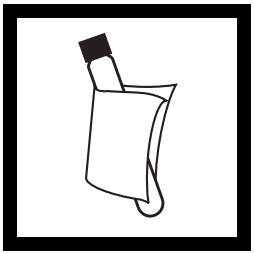


1. Use a TenSette Pipet to add 5.0 mL of sample to a Reactive Phosphorus Test 'N Tube Dilution Vial. Cap and mix.

Note: *Run a reagent blank for this test. Use deionized water in place of the sample. Subtract this result from all test results run with this lot of PhosVer 3 Reagent.*

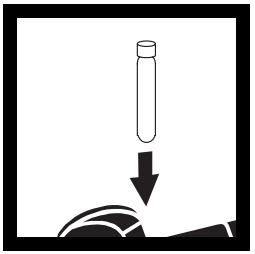


2. Place the COD Vial Adapter into the cell holder.



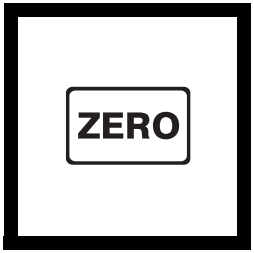
3. Clean the outside of the vial with a towel.

Note: *Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.*



4. Place the vial in the adapter. Place the cover on the adapter.

* Procedure is equivalent to USEPA method 365.2 and Standard Method 4500-PE for wastewater.

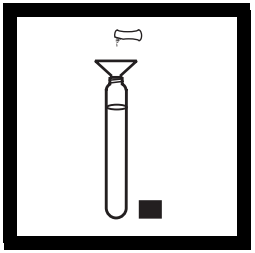


5. Zero the instrument with the vial, using the settings below.

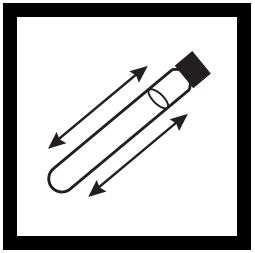
DR/800s
Program No. 82

DR/2010
Program No. 535
890 nm

DR/4000
Program No. 3036
890 nm



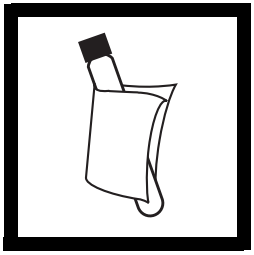
6. Using a funnel, add the contents of one PhosVer 3 Phosphate Powder Pillow to the vial.



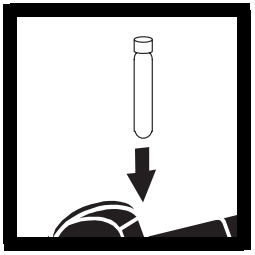
7. Cap the vial tightly and shake for 10-15 seconds.

Note: *The powder will not completely dissolve.*

8. Begin a two minute reaction period.



9. After the timer beeps, wipe the outside of the vial with a towel.



10. Place the prepared sample vial into the adapter. Read the mg/L phosphate.

Sampling and Storage

Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test. The sample should have a neutral pH (6–8 pH) and be at room temperature before adding reagents.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples for up to 48 hours by filtering immediately and storing at 4 °C. Warm to room temperature before analysis.

Accuracy Check

Standard Additions Method

Note: *Clean glassware with 1:1 hydrochloric acid solution. Rinse again with deionized water. Do not use detergents containing phosphates to clean glassware.*

a. Fill three 25-mL graduated mixing cylinders with 25 mL of sample.

b. Snap the neck off a Phosphate Voluette Ampule Standard, 50 mg/L as PO_4^{3-}.

c. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL, respectively, to the three 25-mL aliquots of sample prepared in *step a*. Mix well.

d. Analyze each sample from Step C as described in the procedure. Use 5 mL of the prepared sample for each test. The concentration should increase as follows: 0.2 mg/L, 0.4 mg/L, 0.6 mg/L PO_4^3, respectively.

e. If these increases do not occur, an interference is likely.

Standard Solution Method

To check accuracy, use a 1.0 mg/L Phosphate Standard Solution listed under *Optional Reagents*. Or, prepare by pipetting 2.0 mL of solution from a Voluette Ampule Standard for Phosphate, 50 mg/L as PO_4^{3-}, into a 100-mL Class A volumetric flask. Fill to the line with deionized water. Substitute this standard for the sample and perform the procedure as described. The mg/L PO_4^{3-} reading should be 1.0 mg/L.

Interferences

Large amounts of turbidity may cause inconsistent test results because acid in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.

The following may interfere when present in concentrations exceeding these listed below:

Aluminum	200 mg/L
Chromium	100 mg/L
Copper	10 mg/L
Iron	100 mg/L
Nickel	300 mg/L
Silica	50 mg/L
Silicate	10 mg/L
Sulfide	6 mg/L
Zinc	80 mg/L

Sulfide interference may be removed by oxidation with Bromine Water as follows:

a. Measure 25 mL of sample into a 50-mL beaker.

b. Swirling constantly, add Bromine Water drop-wise until a permanent yellow color develops.

c. Swirling constantly, add Phenol Solution dropwise until the yellow color just disappears. Proceed with *step 3*.

Arsenate interferes at all levels.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.

Sample Disposal Information

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

REQUIRED REAGENTS

Description	Unit	Cat. No.
Reactive Phosphorus Test 'N Tube™ Reagent Set	50 tests	27425-45
Includes: (1) 21060-46, (50) Ortho Phosphate Test 'N Tube™ Dilution Vials*		

Description	Quantity Required Per Test	Unit	Cat. No.
PhosVer® 3 Phosphate Reagent Powder Pillows	1	50/pkg	21060-46
Ortho Phosphate Test 'N Tube™ Dilution Vials	1	50/pkg	*

REQUIRED APPARATUS

Test 'N Tube™ Vials	1	6/pkg	22758-06
COD Vial Adapter, DR/2010	1	each	44799-00
COD Vial Adapter, DR/4000	1	each	48189-00
COD Vial Adapter, DR/800	1	each	48464-00
Funnel, micro	1	each	25843-35
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	varies	5/pkg	21997-96
Test Tube Rack	1-3	each	18641-00

* These items are not sold separately.

OPTIONAL REAGENTS

Description	Unit	Cat. No.
Bromine Water, 30 g/L	29 mL	2211-20
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phenol Solution, 30 g/L	29 mL	2112-20
Phosphate Standard Solution, 1 mg/L as PO_4^{3-}	500 mL	2569-49
Phosphate Standard Solution, Voluette™ Ampule, 50 mg/L as PO_4^{3-}, 10 mL	16/pkg	171-10

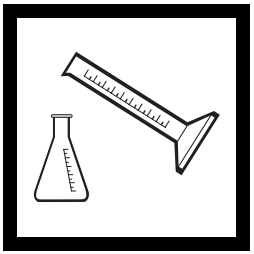
OPTIONAL APPARATUS

Ampule Breaker Kit	each	21968-00
Aspirator, vacuum	each	2131-00
Cylinder, graduated, mixing, 25 mL	each	20886-40
Filter Holder, 47 mm, 300 mL, graduated	each	13529-00
Filter Membrane, 47 mm, 0.45 microns	100/pkg	13530-01
Flask, filtering, 500 mL	each	546-49
pH Indicator Paper, 1 to 11 pH units	5 rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable	each	51700-10
Pipet Filler, Safety Bulb	each	14651-00
Pipet, TenSette®, 0 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 5 mL	each	14515-37
Stopper, No. 7, one hole	6/pkg	2119-07
Tubing, rubber	12 feet	560-19

PHOSPHORUS, TOTAL

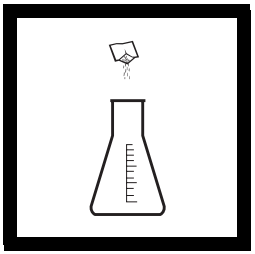
Acid Persulfate Digestion* (Also called Organic and Acid Hydroyzable Phosphorus)

USEPA Accepted for reporting wastewater analysis**

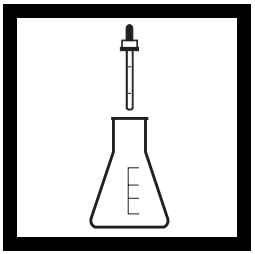


1. Measure 25 mL of sample into a 50-mL (or larger) Erlenmeyer flask using a graduated cylinder.

Note: *Rinse all glassware with 1:1 Hydrochloric Acid Solution. Rinse again with deionized water.*

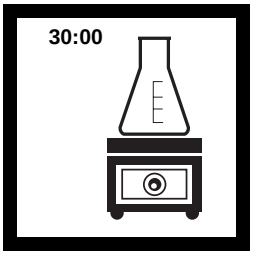


2. Add the contents of one Potassium Persulfate Powder Pillow. Swirl to mix.



3. Add 2.0 mL of 5.25 N Sulfuric Acid Solution.

Note: *Use the 1-mL calibrated dropper provided.*

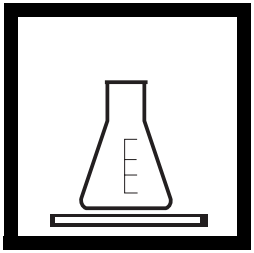


4. Place the flask on a hot plate. Boil gently for 30 minutes.

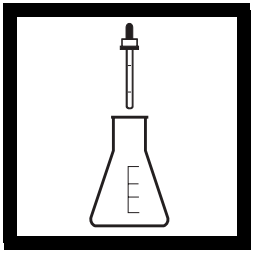
Note: *Concentrate samples to less than 20 mL for best recovery. After concentration maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

** Procedure is equivalent to USEPA Method 365.2 and Standard Method 4500-P B,5 and P E.



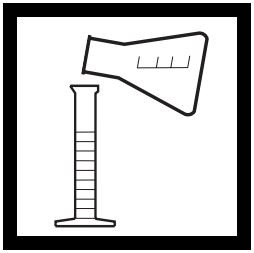
5. Cool the sample to room temperature.



6. Add 2.0 mL of 5.0 N Sodium Hydroxide Solution. Swirl to mix.

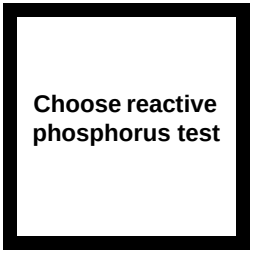
Note: *Use the 1-mL calibrated dropper provided.*

Note: *Adjust the pH to 8.2 for PhosVer 3 method.*



7. Pour the sample into a 25-mL graduated cylinder. Return the volume to 25 mL.

Note: *Use deionized water rinsings from the flask to adjust the volume.*



8. Proceed with a reactive phosphorus method starting at step *3* on page *399*.

Note: *Results of the reactive phosphorus test at this point will include the organic phosphate plus the orthophosphate and the acid-hydrolyzable (condensed) phosphate. The organic phosphate concentration is determined by subtracting results of an acid hydrolyzable phosphorus test from this result. Make sure that both results are in the same units before taking the difference.*

Sampling and Storage

Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples up to 28 days by adjusting the pH to 2 or less with sulfuric acid (about 2 mL per liter) and storing at 4 °C. Warm to room temperature before testing. Adjust the sample to pH 8.2 if using the PhosVer method finish. Correct results for volume additions.

Interferences

For turbid samples, use 50 mL of sample and double the reagent quantities. Use 25 mL of the reacted sample to zero the instrument in the reactive phosphorus procedure. This compensates for any color or turbidity destroyed by this procedure. For alkaline or highly buffered samples it may be necessary to add additional acid in Step 3 to drop the pH of the solution below 1.

Summary of Method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphate by heating with acid and persulfate. Organically bound phosphates are thus determined indirectly by subtracting the result of an acid hydrolyzable phosphorus test from the total phosphorus result.

This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determination of the phosphorus content of the sample. If the ascorbic acid (PhosVer 3) method is used to measure the reactive phosphorus, this method is EPA approved for NPDES reporting.

The following reagents and apparatus are required besides those required for the reactive phosphorus test.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Potassium Persulfate Powder Pillows	1	100/pkg	2451-99
Sodium Hydroxide Solution, 5.0 N	2 mL	100 mL* MDB	2450-32
Sulfuric Acid Solution, 5.25 N	2 mL	100 mL* MDB	2449-32

REQUIRED APPARATUS

Cylinder, graduated, 25 mL	2	each	508-40
Flask, Erlenmeyer, 50 mL	1	each	505-41

OPTIONAL REAGENTS

Hydrochloric Acid, 6 N	500	mL	884-49
Sodium Hydroxide Solution, 5.0 N	1	L	2450-53
Sulfuric Acid, ACS	500	mL	979-49
Water, deionized	4	L	272-56

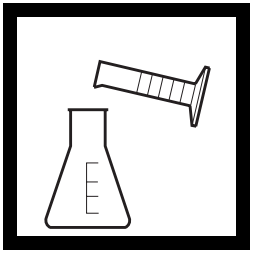
OPTIONAL APPARATUS

Cylinder, graduated, 50 mL		each	508-41
Flask, Erlenmeyer, 125 mL		each	505-43
Hot Plate, 10 cm (4 in.) diameter, 120 Vac		each	12067-01
Hot Plate, 10 cm (4 in.) diameter, 240 Vac		each	12067-02
Pads, cooling, 10 x 10 cm (4 x 4 in.)		each	18376-00
pH Indicator Paper, 1 to 11 pH	5	rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable		each	51700-10

* Contact Hach for larger sizes.

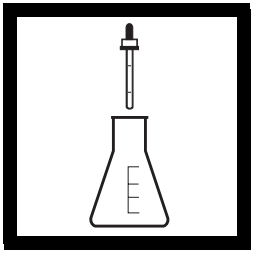
PHOSPHORUS, ACID HYDROLYZABLE

Hydrolysis to Orthophosphate Method*



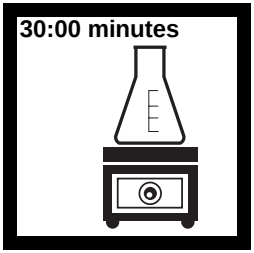
1. Measure 25 mL of sample into a 50-mL (or larger) Erlenmeyer flask using a graduated cylinder.

Note: *Wash all glassware with 6 N hydrochloric acid. Rinse with deionized water.*



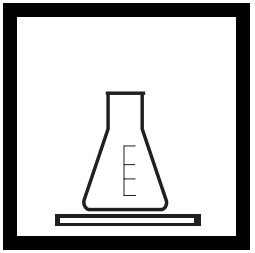
2. Add 2.0 mL of 5.25 N Sulfuric Acid Solution.

Note: *Use the 1-mL calibrated dropper provided.*



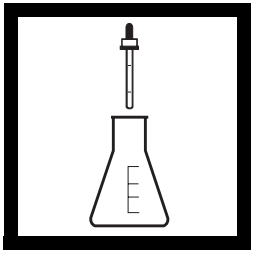
3. Place the flask (the prepared sample) on a hot plate. Boil gently for 30 minutes.

Note: *Concentrate samples to less than 20 mL for best recovery. After concentration, maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.*



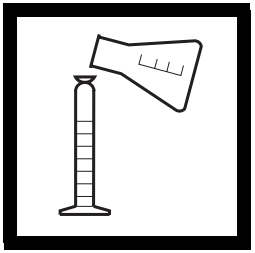
4. Cool the prepared sample to room temperature.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*



5. Add 2.0 mL of 5.0 N Sodium Hydroxide Solution to the prepared sample. Swirl to mix.

Note: *Use the 1-mL calibrated dropper provided.*



6. Pour the prepared sample into a graduated cylinder. Add deionized water rinsings from the flask to return the volume to 25 mL. Proceed with the PhosVer 3 reactive phosphorus test starting with *step 3* on page*399*.

Note: *Results of the reactive phosphorus test at this point will include the orthophosphate plus the acid-hydrolyzable (condensed) phosphate. The condensed phosphate concentration is determined by subtracting the results of a reactive phosphorus test on an untreated sample from this result. Make sure both results are in the same units.*

Sampling and Storage

The most reliable results are obtained when samples are analyzed immediately. If prompt analysis is not possible, samples may be preserved up to 48 hours by cooling to 4 °C (39 °F). Warm to room temperature before testing.

Interferences

If the sample is turbid, use 50 mL of sample and double the reagent volumes. Use 25 mL of the hydrolyzed sample to zero the instrument in the reactive phosphorus procedure. This compensates for any turbidity dissolved by this procedure.

Summary of Method

This procedure lists the necessary steps to convert condensed phosphate forms (meta-, pyro- or other polyphosphates) to reactive orthophosphate before analysis. The procedure uses acid and heat to hydrolyze the sample. Organic phosphates are not converted to orthophosphate by this process, but a very small

fraction may be unavoidably included in the result. Thus, the "acid hydrolyzable" phosphate results are primarily a measure of inorganic phosphorus. This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determination of the phosphorous content of the sample.

The following reagents and apparatus are required in addition to those required for the reactive phosphorus test.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Sodium Hydroxide Solution, 5.0 N	2 mL	100 mL* MDB	2450-32
Sulfuric Acid Solution, 5.25 N	2 mL	100 mL* MDB	2449-32

REQUIRED APPARATUS

Cylinder, graduated, 25 mL	2	each	508-40
Flask, Erlenmeyer, 50 mL	1	each	505-71

OPTIONAL REAGENTS

Hydrochloric Acid, 6 N	500	mL	884-49
Water, deionized	4	L	272-56

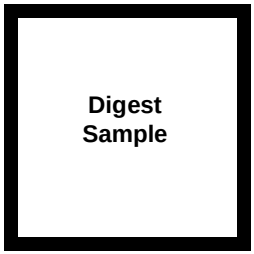
OPTIONAL APPARATUS

Cylinder, graduated, 50 mL		each	508-41
Flask, Erlenmeyer, 125 mL		each	505-43
Hot Plate, 4" diameter, 120 Vac		each	12067-01
Hot Plate, 10 cm (4 in.) diameter, 240 Vac		each	12067-02
Pad, cooling, 10 x 10 cm (4 x 4 in.		each	18376-00
pH indicator Paper, 1 to 11 pH	5	rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable		each	51700-10

* Contact Hach for larger sizes.

PHOSPHORUS, TOTAL

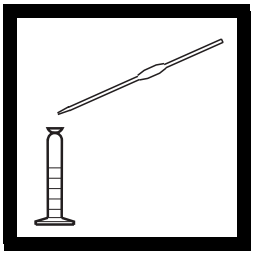
PhosVer 3 Ascorbic Acid Method* USEPA Accepted for wastewater analysis reporting
Range: Liquids- 0.16–12500 mg/L; Solids- 12.5–125000 mg/kg



1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

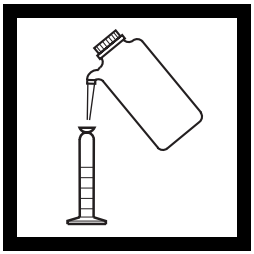
Note: *If sample cannot be analyzed immediately after collection, see* Sampling and Storage *following these steps.*

Note: *The Digesdahl is not a USEPA approved digestion. Samples digested using the Digesdahl may not be used for reporting. For reporting purposes, use the Acid Persulfate procedure, Hach Method 8190.*

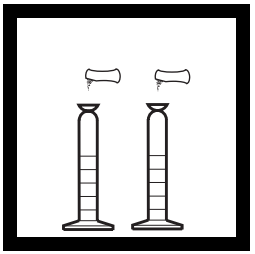


2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 10-mL mixing cylinder. If the aliquot is more than 0.2 mL, adjust the pH (see the note below). Dilute to the 10-mL mark with deionized water.

Note: *Adjust the pH to 8.2 for the PhosVer method finish.*



3. Fill a second 10-mL graduated mixing cylinder with deionized water (the blank).

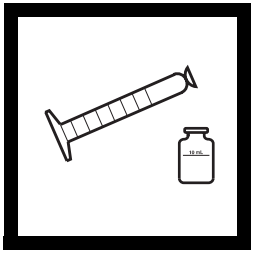


4. Add the contents of one PhosVer 3 Reagent Powder Pillow (for 10-mL samples) to each cylinder. Stopper the cylinders and invert to mix.

Note: *A blue color will develop if phosphate is present.*

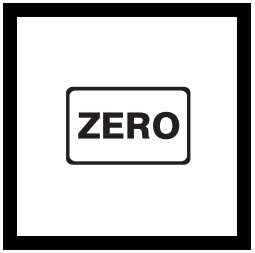
Note: *Not all the powder will dissolve.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater.* Procedure is equivalent to USEPA Method 365.2 and Standard Method 4500-P-E for wastewater.



5. Pour the contents of each cylinder into separate 10-mL sample cells.

6. Begin a two minute reaction period.

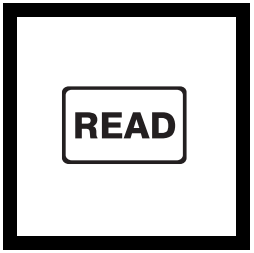


7. After the timer beeps, place the blank in the cell compartment. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 79

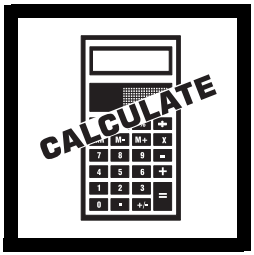
DR/2010
Program No. 490
890 nm

DR/4000
Program No. 3025
890 nm



8. Place the sample in the cell compartment. Read the mg/L phosphate.

Note: *The final solution will contain molybdenum. Use proper disposal procedures.*



9. Use the equation below the phosphorus Sample and Analysis Volume Tables to calculate the true phosphate concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample.The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected PO_4 Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.16-7.8	40.0	8.00	10 mL
0.7-31	20.0	4.00	10 mL
2.5-125	10.0	2.00	10 mL
25-1250	5.00	0.40	10 mL
250-12500	1.00	0.20	10 mL

Solids

Expected PO_4 Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
12.5-625	0.500	8.00	10 mL
32-1560	0.400	4.00	10 mL
85-4150	0.300	2.00	10 mL
625-31250	0.200	0.40	10 mL
2500-125000	0.100	0.20	10 mL

Calculation For Final Concentration:

$$\frac{A \times 1000}{B \times C} = \text{mg/L or mg/kg Total Phosphorus}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples for up to 48 hours by filtering immediately and storing samples at 4 °C.

Accuracy Check

Standard Additions Method

a. Perform the phosphorus method and note the analysis volume and the mg/L phosphate of the sample.

b. Pipet the same analysis volume into three 10-mL graduated mixing cylinders.

c. Snap the neck off a Phosphate Voluette Ampule Standard Solution, 50 mg/L PO_4^{3-}.

d. Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard, respectively, to the three mixing cylinders. Dilute to 10 mL. Stopper each and mix thoroughly.

e. Analyze each standard addition sample as described in the procedure. The phosphate concentration should increase 0.5 mg/L for each 0.1 mL of standard added.

f. If these increases do not occur, an interference is likely.

Standard Solution Method

Prepare a 2.0 mg/L PO_4^{3-} standard solution by pipetting 4.0 mL of Phosphate Standard Solution, 50 mg/L as PO_4^{3-}, into a 100-mL volumetric flask. Dilute to volume with deionized water. Stopper and invert to mix. Use this solution in place of the sample in the procedure to ensure the accuracy of the test. The mg/L PO_4^{3-} reading should be 2 mg/L.

Interferences

Large amounts of turbidity may cause inconsistent results in the phosphate tests because the acid present in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. For highly turbid or colored samples, add the contents of one Phosphate Pretreatment Powder Pillow to 10 mL of sample. Mix well. Use this solution to zero the instrument instead of a reagent blank.

The PhosVer 3 Phosphate reagent Powder Pillows should be stored in a cool, dry environment.

The following may interfere when present in concentrations exceeding these listed below:

Aluminum	200 mg/L
Chromium	100 mg/L
Copper	10 mg/L*
Iron	100 mg/L*
Nickel	300 mg/L
Silica	50 mg/L
Silicate	10 mg/L
Zinc	80 mg/L

* Copper and iron may be measured by diluting the sample 1:20 and using Water Quality Copper Test Strips (Cat. No. 27451-25) or Water Quality Iron Test Strips (Cat. No. 274530-25).

Arsenate interferes.

Summary of Method

Orthophosphate reacts with molybdate in an acid medium to produce a Phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
PhosVer® 3 Phosphate Reagent Powder Pillows			
10 mL sample size	2	100/pkg	21060-69
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS

Cylinder, graduated, mixing, 10 mL	2	each	20886-38

Select one or more based an sample amount and analysis volume:

Pipet, volumetric, Class A, 8.0 mL	1	each	14515-08
Pipet, volumetric, Class A, 4.0 mL	1	each	14515-04
Pipet, volumetric, Class A, 2.0 mL	1	each	14515-36
TenSette Pipet, 0.1-1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01	1	50/pkg	21856-96

OPTIONAL REAGENTS

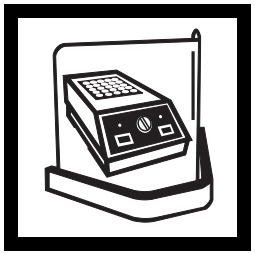
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884-49
Phosphate Pretreatment Powder Pillows	100/pkg	14501-99
Phosphate Standard Solution, Voluette ampule, 50 mg/L as PO_4^{3-}, 10 mL	16/pkg	171-10
Phosphate Standard Solution, 50 mg/L as PO_4^{3-}	500 mL	171-49
Phosphate Standard Solution, 1 mg/L as PO_4^{3-}	500 mL	2569-49
Water Quality Test Strips, total iron	25/pkg	27453-25
Water Quality Test Strips, total copper	25/pkg	27451-25

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Filter Holder, 47 mm, 300 mL, graduated	each	13529-00
Filter, membrane, 47 mm, 0.45 microns	100/pkg	13530-01
Flask, filtering, 500 mL	each	546-49
Flask, volumetric, Class A, 100 mL	each	14574-42
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
pH Meter, *sens**ion***™***1**, portable	each	51700-10
Pipet, 2 mL serological	each	532-36
Pipet, TenSette®, 1.0 to 10.0 mL	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	50/pkg	21997-96
Pipet Tips, for 19700-10 TenSette® Pipet	250/pkg	21997-25
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 Tensette® Pipet	50/pkg	21856-96
Stopper, No. 7, one hole	6/pkg	2119-07
Thermometer, -10 to 110 °C	each	1877-01
Tubing, rubber	12 feet	560-19

PHOSPHORUS, TOTAL

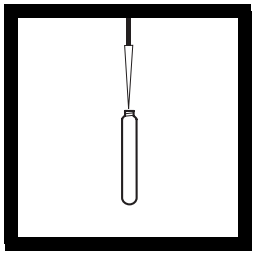
PhosVer 3 Test 'N Tube With Acid Persulfate Digestion*
USEPA accepted for reporting wastewater analysis**
Range: 0.00–3.50 mg/L PO_4^{3-}



1. Turn on the COD Reactor. Heat to 150 °C. Place the plastic shield in front of the reactor.

Note: *Ensure safety devices are in place to protect the analyst from splattering should leakage occur.*

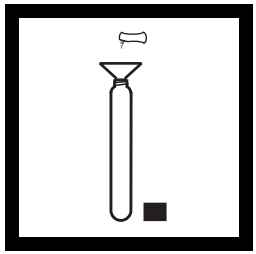
Note: *See COD Reactor manual for temperature adjustment instructions.*



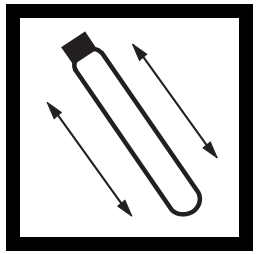
2. Add 5 mL of sample to a Total and Acid Hydrolyzable Test Vial.

Note: *For unpreserved samples with extreme pH, see* Interferences *following these steps.*

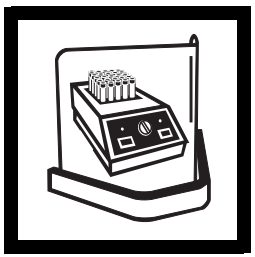
Note: *Run a reagent blank for this test using deionized water in place of the sample. Subtract the blank value from tests results. Repeat for each new lot of reagents.*



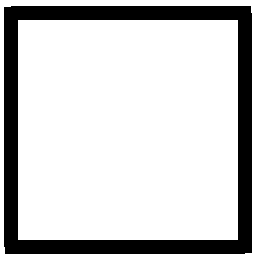
3. Using a funnel, add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.



4. Cap tightly and shake to dissolve.

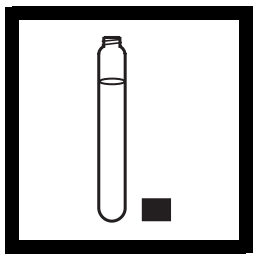


5. Heat the vial for 30 minutes.

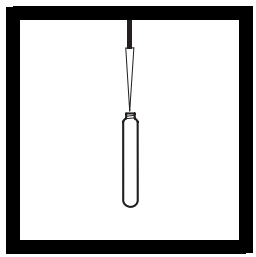


6. Carefully remove the vial from the reactor. Place it in a test tube rack and allow to cool to room temperature.

Note: *Tubes will be hot.*



7. After cooling, remove the cap from the vial.



8. Add 2 mL of 1.54 N Sodium Hydroxide Standard solution to the vial.

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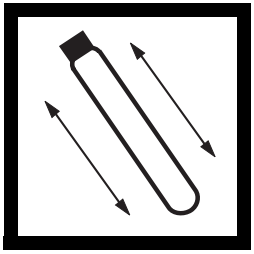
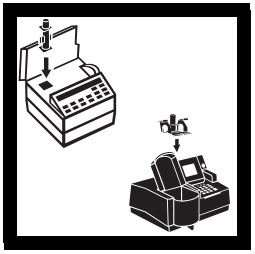
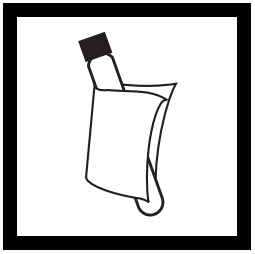
* Adapted from *Standard Methods for the Examination of Water and Wastewater.*
** Procedure is equivalent to USEPA method 365.2 and Standard Method 4500-P B, 5 and P.E.



9. Cap tightly and shake to mix.

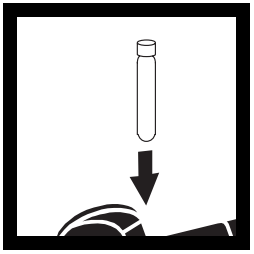


10. Place the COD Vial Adapter into the cell holder.

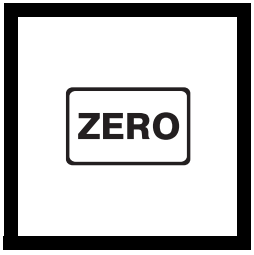


11. Clean the outside of the vial with a towel.

Note: *Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.*



12. Place the sample vial in the adapter. Place the cover on the adapter.

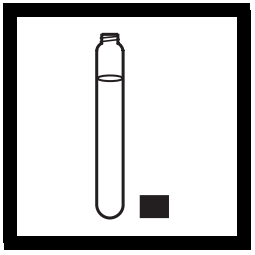


13. Zero the instrument with the vial, using the settings below.

DR/800s
Program No. 82

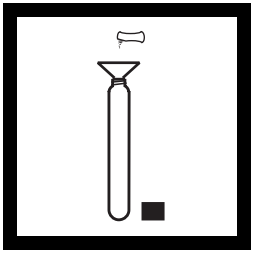
DR/2010
Program No. 535
890 nm

DR/4000
Program No.3036
890 nm

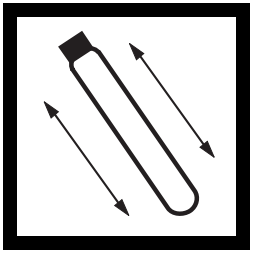


14. Remove the vial from the instrument.

Note: *For multiple samples from the same source, zero only on the first sample. Read the remaining samples after adding the PhosVer 3 reagent. Subtract the PhosVer 3 reagent blank value from each reading.*



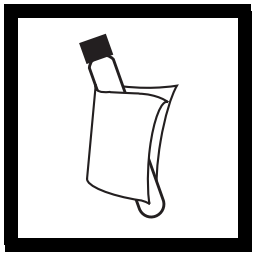
15. Using a funnel, add the contents of one PhosVer 3 Phosphate Reagent Powder Pillow to the vial.



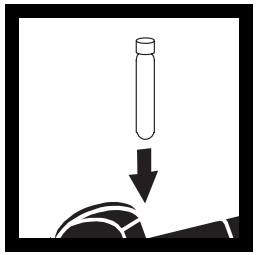
16. Cap tightly and shake for 10-15 seconds.

Note: *The powder will not completely dissolve.*

17. Begin a two-minute reaction period.



18. After the timer beeps, wipe the outside of the vial with a towel.



19. Place the prepared sample vial into the adapter. Read the mg/L phosphate.

IMPORTANT NOTE:

The test range for total phosphate is limited to 0 to 3.5 mg/L PO_4^{3-}. Values above 3.5 mg/L may be used to estimate dilution ratios, but should NOT be used for reporting purposes. If a test overranges, dilute the sample and repeat the digestion and colorimetric testing for accurate results.

Sampling and Storage

Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve the sample for up to 28 days by adjusting the pH to 2 or less with concentrated sulfuric acid (about 2 mL per liter) and refrigerating at 4 °C. Before analysis, warm to room temperature and adjust the pH to 7.

Correct results for volume additions.

Accuracy Check

Standard Additions Method

a. Fill three 25 mL graduated mixing cylinders with 25 mL of sample.

b. Snap the neck off a Phosphate Voluette Ampule Standard, 50 mg/L as PO_4^{3-}.

c. Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL, respectively, to the three 25-mL aliquots of a water sample. Mix well.

d. Analyze samples from Step C as described in the procedure; use 5 mL of the prepared sample for each test. The concentration should increase as follows: 0.20 mg/L, 0.40 mg/L, 0.60 mg/L PO_4^{3-}, respectively.

e. If these increases do not occur, an interference is likely.

Standard Solution Method

To check accuracy, use a 1.0 mg/L Phosphate Standard Solution listed under Optional Reagents. Or, this can be prepared by pipetting 2 mL of solution from a Voluette Ampule Standard for Phosphate, 50 mg/L as PO_4^{3-}, into a Class A 100-mL volumetric flask. Dilute to the mark with deionized water. Substitute this standard for the sample and perform the procedure as described. The mg/L PO_4^{3-} reading should be 1.0 mg/L.

Interferences

Large amounts of turbidity may cause inconsistent results in the test because the acid present in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.

The PhosVer 3 Phosphate Reagent Powder Pillows should be stored in a cool, dry environment.

The following may interfere when present in concentrations these listed below:

Aluminum	200 mg/L
Chromium	100 mg/L
Copper	10 mg/L*
Iron	100 mg/L*
Nickel	300 mg/L
Silica	50 mg/L
Silicate	10 mg/L
Sulfate	90 mg/L
Zinc	80 mg/L

* Copper and iron may be measured by diluting the sample 1:20 and using Water Quality Copper Test Strips (Cat. No. 27451-25) or Water Quality Iron Test Strips (Cat. No. 274530-25).

Arsenate interferes at all levels.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.

Sample Disposal Information

The phosphate vials will contain molybdenum, which is a regulated substance. Also, the pH of these solutions is less than 2. Follow all Federal, state and local regulations for disposal.

Summary of Method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

REQUIRED REAGENTS

Description	Cat. No.
Total Phosphorus Reagent Set, (50 tests)	27426-45

Includes: 50 PhosVer® 3 Phosphate Powder Pillows, 50 Potassium Persulfate for Phosphonate Powder Pillows, 100 mL 1.54 N Sodium Hydroxide, 50 Total and Acid Hydrolyzable Test Vials.

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
COD Vial Adapter, DR/2010	1	each	44799-00
COD Vial Adapter, DR/4000	1	each	48189-00
COD Vial Adapter, DR/800	1	each	48464-00
COD Reactor, 120/240 Vac (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 120/240 Vac (Europe)	1	each	45600-02
Funnel, micro	1	each	25843-35
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1-3	each	18641-00
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	2	50/pkg	21997-96

OPTIONAL REAGENTS

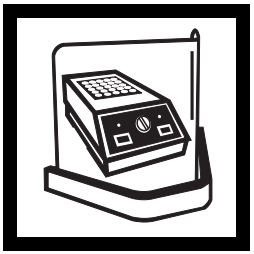
Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500	mL	884-49
Phosphate Standard Solution, 1 mg/L as PO_4^{3-}	500	mL	2569-49
Phosphate Standard Solution, Voluette™ Ampule, 50 mg/L as PO_4^{3-}, 10 mL	16	/pkg	171-10
Sodium Hydroxide Standard Solution, 5 N	1	L	2450-53
Sulfuric Acid, ACS	500	mL	979-49
Sulfuric Acid Standard Solution, 1.000 N	1	L	1270-53
Total Acid Hydrolyzable Phosphorus Reagent Set, 50 tests	each		27427-45
Water, deionized	4	L	272-56
Water Quality Test Strips, total copper	25	/pkg	27451-25
Water Quality Test Strips, total iron	25	/pkg	27453-25

OPTIONAL APPARATUS

Cylinder, graduated, mixing, 25 mL	each		20886-40
Flask, volumetric, Class A, 100 mL	each		14574-42
pH Indicator Paper, 1 to 11 pH units	5 rolls	/pkg	391-33
pH Meter, *sens**ion**™1*, portable	each		51700-10
Pipet Filler, safety bulb	each		14651-00
Pipet, volumetric, Class A, 5.00 mL	each		14515-37
Pipet, volumetric, Class A, 2.00 mL	each		14515-36

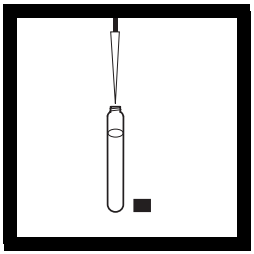
PHOSPHORUS, TOTAL, High Range

Molybdovanadate Method, Test 'N Tube Procedure*
Range: 0.0 to 100.0 mg/L PO_4^{3-}

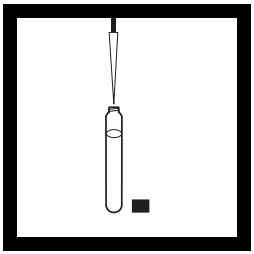


1. Turn on the COD Reactor. Heat to 150 °C. Place the plastic shield in front of the reactor.

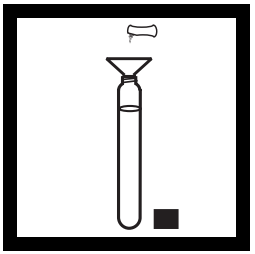
Note: *Ensure safety devices are in place to protect the analyst if splattering or leakage occurs.*



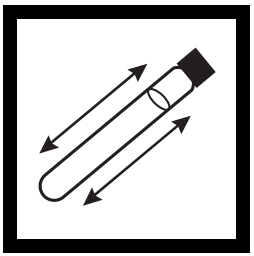
2. Use a TenSette Pipet to add 5.0 mL of deionized water to a Total and Phosphorus Test Vial (the blank).



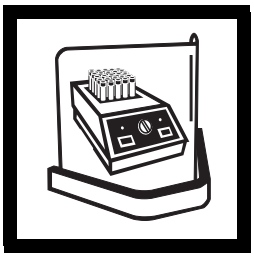
3. Use a TenSette Pipet to add 5.0 mL of sample to a Total and Phosphorus Test Vial (the sample).



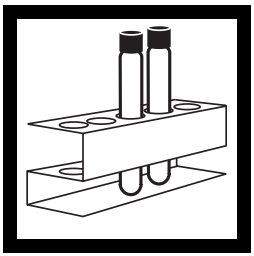
4. Using a funnel, add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to each vial.



5. Cap tightly and shake to dissolve.

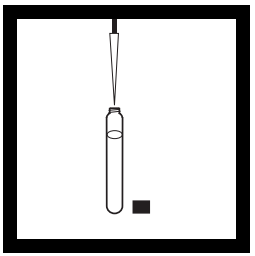


6. Place the vials in the COD reactor. Heat for 30 minutes at 150 °C.



7. Carefully remove the vials from the reactor. Place them in a test tube rack and allow to cool to 18–25 °C.

Note: *Tubes will be hot.*

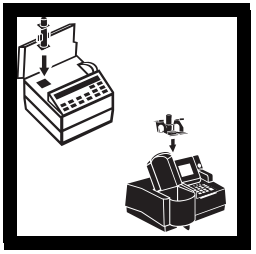


8. Use a TenSette Pipet to add 2.0 mL of 1.54 N sodium hydroxide to each vial.

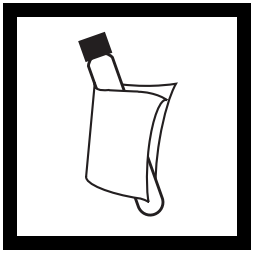
Cap and invert to mix.

* Adapted from *Standard Methods for the Examination of Water and Wastewater.*

9. Begin a seven minute reaction period

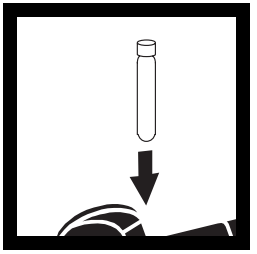


10. Place the COD Vial Adapter into the cell holder.

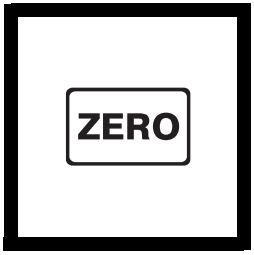


11. Clean the outside of the vials with a towel.

Note: *Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.*



12. Place the sample vial in the adapter. Place the cover on the adapter.

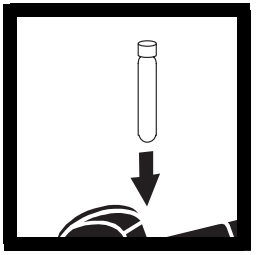


13. Zero the instrument with the vial, using the settings below.

DR/800s
Program No. 87

DR/2010
Program No. 541
420 nm

DR/4000
Program No. 3040
420 nm



14. Place the prepared sample vial into the adapter. Read the mg/L phosphate.

Sampling and Storage

Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric Acid Solution and rinsed with deionized water. Do not use commercial detergents containing phosphate for cleaning glassware used in this test.

Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve the sample for up to 28 days by adjusting the pH to 2 or less with concentrated H_2SO_4 (about 2 mL per liter) and storing at 4 °C. Warm the sample to room temperature and neutralize with 5.0 N NaOH before analysis. Correct results for volume additions.

Accuracy Check

Note: *Clean glassware with 1:1 hydrochloric acid solution. Rinse again with deionized water. Do not use detergents containing phosphates to clean glassware.*

Standard Additions Method

a. Fill each of three 10-mL graduated mixing cylinders with 10 mL of sample.

b. Snap the neck off a 10-mL Voluette Ampule of Phosphate Standard Solution, 500 mg/L as PO_4^{3-}.

c. Use a TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL, respectively, to the three 10-mL aliquots of the water sample prepared in *step a* Mix well.

d. Analyze samples from *step c* as described in the procedure. Use 5.0 mL of the prepared sample for each test. The concentration should increase: 5 mg/L, 10 mg/L, and 15 mg/L PO_4^{3-}, respectively.

e. If these increases do not occur, an interference is likely.

Standard Solution Method

To check accuracy, prepare a 80-mg/mL standard by pipetting 8.0 mL of solution from a Voluette Ampule of Phosphate Standard Solution, 500 mg/L as PO_4^{3-} into an acid-cleaned, Class A, 50-mL volumetric flask. Dilute to the mark with deionized water. Substitute this standard for the sample and perform the procedure as described.

Interferences

Large amounts of sample turbidity may cause inconsistent results in the test because the acid present in the reagents may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.

The following may interfere when present in concentrations exceeding these listed below:

Interfering Substance	Interference Level and Treatment
Arsenate	Causes positive interference if the sample is heated.*
Iron, ferrous	Blue color caused by ferrous iron does not interfere if iron concentration is less than 100 mg/L.
Molybdate	Causes negative interference above 1000 mg/L.
Silica	Causes positive interference if the sample is heated.*
Extreme pH or highly buffered samples	May exceed buffering capacity of the reagents. See *pH Interferences* in *Section I* of the *DR/2010 Procedures Manua*l. Samples may require pretreatment. Sample pH should be about 7.
Fluoride, thorium, bismuth, thiosulfate or thiocyanate	Cause negative interference
Temperature	Cold samples — cause a negative interference. For example, a sample at a temperature of 13 °C (55 °F) has a reaction time of 15 minutes. Hot samples — cause a positive interference. For example, a sample at a temperature of 33 °C (91 °F) has a reaction time of 2 minutes.
The following do not interfere in concentrations up to 1000 mg/L: Pyrophosphate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, salicylate, Al^{3+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Li^{+}, Na^{+}, K^{+}, NH_4^{+}, Cd^{2+}, Mn^{2+}, NO_3^{-}, NO_2^{-}, SO_4^{2-}, SO_3^{2-}, Pb^{2+}, Hg^{+}, Hg^{2+}, Sn^{2+}, Cu^{2+}, Ni^{2+}, Ag^{+}, U^{4+}, Zr^{4+}, AsO_3^{-}, Br^{-}, CO_3^{2-}, ClO_4^{-}, CN^{-}, IO_3^{-}, SiO_4^{4-}.	

* Gentle warming of the sample to reach room temperature will not cause this substance to interfere.

Sample Disposal Information

The final samples will contain molybdenum. In addition, the final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Consult the Material Safety Data Sheet for information specific to the reagents used.

Summary of Method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid forms. The intensity of the yellow color is proportional to the phosphate concentration.

REQUIRED REAGENTS

Description	Cat. No.
Total High Range Phosphorus Test 'N Tube™ Reagent Set, 50 vials	27672-45
Includes: (50) Total and Phosphorus Test Vials*, (2) 272-42, (1) 20847-66 (1) 20760-26, (1) 27430-42	

Description	Quantity Required Per Test	Unit	Cat. No.
Molybdovanadate Reagent	0.5 mL	25 mL	20760-26
Potassium Persulfate Powder Pillows	1	50/pkg	20847-66
Sodium Hydroxide Solution, 1.54 N	2 mL	100 mL	27430-42
Total and Phosphorus Test Vials	1	50/pkg	*
Water, deionized	100 mL	varies	272-42

* These items are not sold separately.

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
COD Reactor, 115/230 Vac (U.S.A. and Canada)	1	each	45600-00
COD Reactor, 115/230 Vac (Europe)	1	each	45600-02
COD Vial Adapter, DR/2010	1	each	44799-00
COD Vial Adapter, DR/4000	1	each	48189-00
COD Vial Adapter, DR/800	1	each	48464-00
Dropper, LDPE, 0.5–1.0 mL	1	20/pkg	21247-20
Pipet, TenSette®, 1 to 10 mL	1	each	19700-10
Pipet Tips, for 19700-10 TenSette® Pipet	2	50/pkg	21997-96
Safety Shield, laboratory bench	1	each	50030-00
Test Tube Rack	1–3	each	18641-00

OPTIONAL REAGENTS

Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500	mL	884-49
Phosphate Standard Solution, PourRite™ ampule, 500 mg/L as PO_4^{3-}, 2 mL	20	/pkg	14242-20
Phosphate Standard Solution, Voluette™ Ampule, 500 mg/L as PO_4^{3-}, 10 mL	16	/pkg	14242-10
Sodium Hydroxide Standard Solution, 5.0 N	1	L	2450-53
Sulfuric Acid, ACS, concentrated	500	mL	979-49

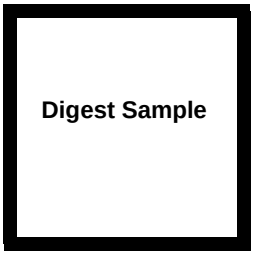
OPTIONAL APPARATUS

Ampule Breaker Kit		each	21968-00
Aspirator, vacuum		each	2131-00
Cylinder, graduated, mixing, 10 mL (3 required)		each	20886-38
Filter Holder, 47 mm, 300 mL, graduated		each	13529-00
Filter, membrane, 47 mm, 0.45 microns	100	/pkg	13530-01
Flask, filtering, 500 mL		each	546-49
Flask, volumetric, Class A, 50 mL		each	14574-41
pH Indicator Paper, 1 to 11 pH units	5	rolls/pkg	391-33
pH Meter, *sens**ion**™1*, portable		each	51700-10
Pipet Filler, Safety Bulb		each	14651-00
Pipet, TenSette®, 0.1 to 1.0 mL		each	19700-01
Pipet Tips, for 19700-01	50	pkg	21856-96
Pipet, volumetric, Class A, 8.00 mL		each	14515-08
Stopper, No. 7 one hole	6	/pkg	2119-07
Tubing, rubber	12	feet	560-19

POTASSIUM

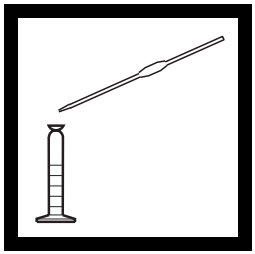
Tetraphenylborate Method
Range: Liquids- 4–30000 mg/L; Solids- 250–350000 mg/Kg

1. This procedure requires a user-entered calibration before sample measurement. See the User-Calibration Section in the DR/2010 or DR/4000 Spectrophotometer instrument manual and *Preparing Calibration Standards* on page *423*.



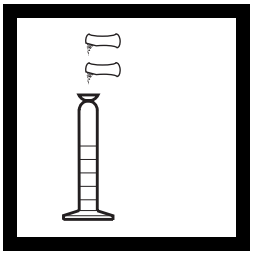
2. Select sample amount from tables following these steps. Digest the sample according to the procedure in *section 3*.

Note: *If sample cannot be analyzed shortly after sampling, see* Sampling and Storage *following these steps.*

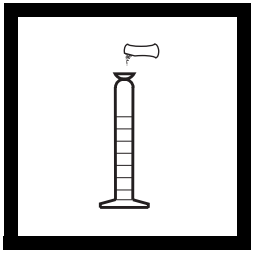


3. Use the analysis volume in the same table. Pipet the sample analysis volume into a 25-mL mixing cylinder. If the aliquot is more than 0.5 mL, adjust the pH according to the instruction following the digestion method.

Note: *FIlter highly colored or turbid samples. Use the filtered sample in this step and step 9.*



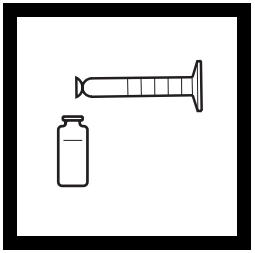
4. Add the contents of one Potassium 1 Reagent Pillow to the sample. Add the contents of one Potassium 2 Reagent Pillow to the sample. Stopper. Invert several times to mix.



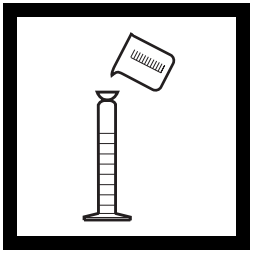
5. After the solution clears, add the contents of one Potassium 3 Reagent Pillow Stopper. Shake for 30 seconds.

Note: *A white turbidity will form if potassium is present.*

6. Begin a three-minute reaction period.

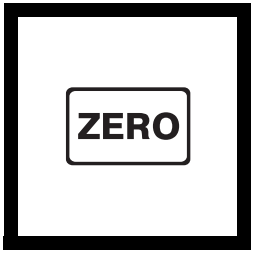


7. Pour the solution from the cylinder into a sample cell (the prepared sample).



8. When the timer beeps, fill a second sample cell (the blank) with 25 mL of sample. Place it into the cell holder.

Note: *For clear samples with low turbidity, use a reagent blank to zero the instrument instead of a sample blank.*

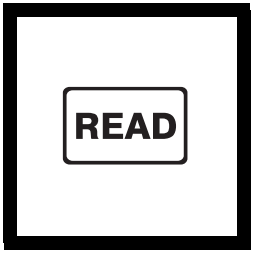


9. Zero the instrument with the blank, using the settings below.

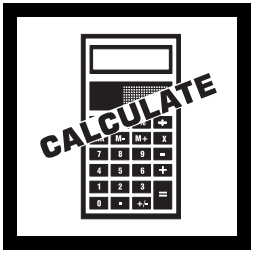
DR/800s
Program No. NA

DR/2010
Program No. is
User Entered
650 nm

DR/4000
Program No. is
User Entered
650 nm



10. Place the sample in the cell holder. Read the %T and determine the mg/L potassium from the calibration curve.



11. Use the equation below the Potassium Sample and Analysis Volume Tables to calculate the true potassium concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected K Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
4–20	40.0	20.0	25 mL
15–80	20.0	10.0	25 mL
60–300	10.0	5.00	25 mL
200–1000	5.0	3.00	25 mL
600–3000	5.00	1.00	25 mL
2000–10000	3.0	0.50	25 mL
6000–30000	1.00	0.50	25 mL

Solids

Expected K Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
250–1750	0.500	20.0	25 mL
625–4375	0.400	10.0	25 mL
1680–11600	0.300	5.00	25 mL
12500–87500	0.200	1.00	25 mL
50000–350000	0.100	0.50	25 mL

Calculation For Final Concentration:

$$\frac{A \times 2500}{B \times C} = \text{mg/L or mg/kg Total K}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-washed plastic bottles. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples may be stored at least six months at room temperature. Adjust the pH to 4 to 5 with 5.0 N sodium hydroxide before analysis. Do not measure pH in the sample container with a pH electrode, as this will introduce potassium from the filling solution. Use pH paper or pour off sample and test pH in a separate beaker. Correct the test result for volume additions.

Accuracy Check

Standard Addition Method

a. Perform the potassium procedure and note the analysis volume used and the mg/L potassium.

b. Pipet the same analysis volume into three 25-mL graduated mixing cylinders.

c. Snap the neck off a Potassium Voluette Ampule Standard Solution, 250 mg/L.

d. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard to three 25-mL samples. Mix each thoroughly.

e. Analyze each sample as described above. The potassium concentration should increase 1.0 mg/L for each 0.1 mL of standard added.

f. If these increases do not occur, an interference is likely.

Standard Solution Method

Check accuracy with a 5.0 mg/L Potassium Standard Solution (see *OPTIONAL REAGENTS*). Or, prepare this standard by diluting 5.00 mL of a 1000-mg/L Potassium Standard Solution to one liter with deionized water.

Preparing Calibration Standards

Make calibration standards using a 100 mg/L Potassium Standard Solution and Class A 100-mL volumetric flasks and pipets. Use at least five standards prepared according to the following table. Invert each flask to mix.

Table 1 Preparing Standards

Final Concentration (mg/L)	Volume of 100-mg/L Standard (mL)	Volume of Deionized Water (mL)
0.0	0	100
1.0	1.0	100
2.0	2.0	100
3.0	3.0	100
4.0	4.0	100
5.0	5.0	100
6.0	6.0	100
7.0	7.0	100
8.0	8.0	100

Interferences

The following ions do not interfere below the concentration shown:

Substance	Level Tested
Ammonium Nitrogen	15 mg/L as N
Calcium	7000 mg/L as $CaCO_3$
Chloride	15,000 mg/L*
Magnesium	6000 mg/L as $CaCO_3$

* Monitor chloride by diluting sample 1:5 and using Water Quality Test strips for High Range Chloride (27513-40).

Summary of Method

Potassium in the sample combines with sodium tetraphenylborate to form potassium tetraphenylborate, an insoluble white solid. The amount of turbidity produced is proportional to the potassium concentration.

REQUIRED REAGENTS

Description	Cat. No.
Potassium Reagent Set (100 tests)	24591-00

Includes: (4) 14321-98, (4) 14322-98, (1) 14323-99

Description	Quantity Required Per Test	Unit	Cat. No.
Potassium 1 Reagent Pillows	1 pillow	25/pkg	14321-98
Potassium 2 Reagent Pillows	1 pillow	25/pkg	14322-98
Potassium 3 Reagent Pillows	1 pillow	100/pkg	14323-99
Potassium Standard Solution, 100 mg/L K	varies	500 mL	23517-49
Water, deionized	varies	4L	272-56

Select one or more based on sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

REQUIRED APPARATUS

Clippers, for opening powder pillows	1	each	968-00
Cylinder, mixing, graduated, 25 mL	1	each	1896-40

OPTIONAL REAGENTS

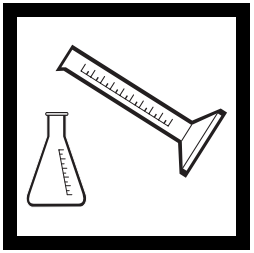
Potassium Standard Solution, 5 mg/L	500 mL		20583-49
Potassium Standard Solution, 1000 mg/L	100 mL		22404-42
Potassium Standard Solution, Voluette™ Ampule, 250 mg/L, 10 mL	16/pkg		14790-10
Quantab Titrators for High Range Chloride	40/pkg		27513-40

OPTIONAL APPARATUS

Ampule Breaker Kit	each		21968-00
Flask, volumetric, 250 mL, Class A	each		14574-46
Graph Paper, semilogarithmic, single cycle	100/pkg		21108-00
Pipet, TenSette®, 0.1 to 1.0 mL	each		19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg		21856-96
Pipet, volumetric, Class A, 1.00 mL	each		14515-35
Pipet, volumetric, Class A, 2.00 mL	each		14515-36
Pipet, volumetric, Class A, 3.00 mL	each		14515-03
Pipet, volumetric, Class A, 4.00 mL	each		14515-04

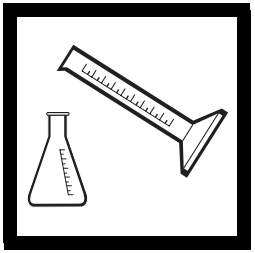
SELENIUM

Diaminobenzidine Method (distillation required for total selenium analysis)
Range: 0–1.00 mg/L

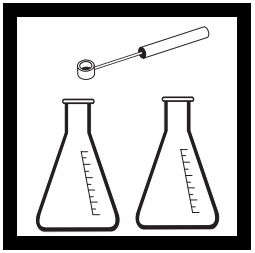


1. Measure 100 mL of deionized water into a 500-mL Erlenmeyer flask (label the flask "blank").

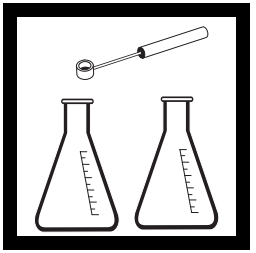
Note: *Selenium present as Se^{2-} or Se^{6+} is not detected unless the sample is distilled. See Distillation following these steps. Use the distillate in step 2.*



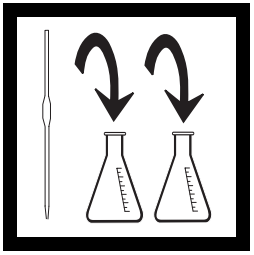
2. Measure 100 mL of sample into a second 500-mL Erlenmeyer flask (label the flask "sample").



3. Add a 0.2-g scoop of TitraVer Hardness Reagent to each flask. Swirl to mix.

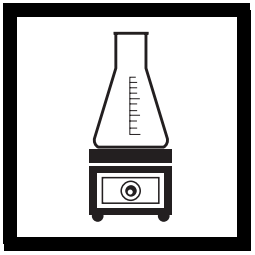


4. Add a 0.05-g scoop of diaminobenzidine tetrahydrochloride to each flask. Swirl to mix.



5. Add 5.0 mL of Buffer Solution, sulfate type, pH 2.0, to each flask. Swirl to mix.

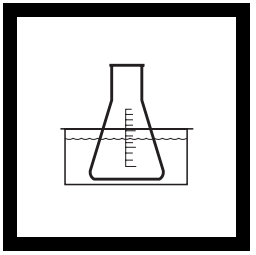
Note: *If the sample has been distilled, omit the Buffer Solution. Adjust the distillate pH to 2.7 (±0.2 pH) using 5 N Sodium Hydroxide Standard Solution. Adjust the blank to the same pH value using 5.25 N Sulfuric Acid Standard Solution.*



6. Heat each flask on a hot plate or over a flame, bringing the contents to a gentle boil.

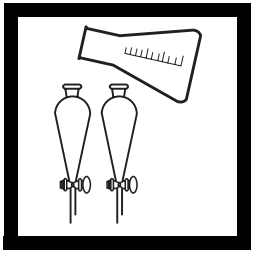
7. Begin a five-minute reaction period. Continue to boil the contents gently during this time period.

Note: *A yellow color will develop if selenium is present.*

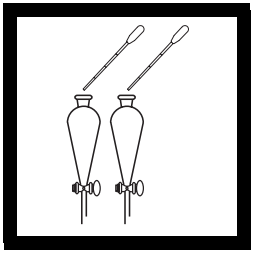


8. When the timer beeps, remove both flasks. Cool to room temperature using a water bath.

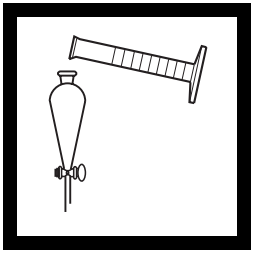
Note: *Do not boil more than one minute after the timer beeps.*



9. Transfer the contents of each flask to separate 250-mL separatory funnels. Label the funnels “blank” and “sample”.



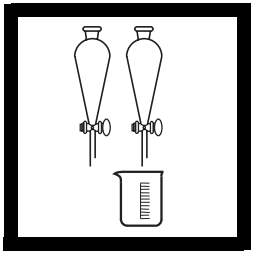
10. Add 2.0 mL of 12 N Potassium Hydroxide Standard Solution to each funnel using a calibrated 1.0-mL plastic dropper. Stopper. Shake each funnel to mix.



11. Add 30 mL of toluene to each funnel. Stopper. Shake each funnel vigorously for 30 seconds.

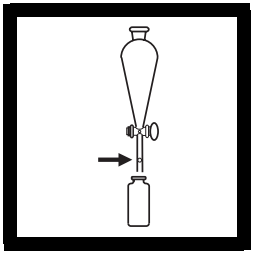
Note: *Use toluene only with adequate ventilation.*

12. Begin a three-minute reaction period.



13. When the timer beeps, drain the lower water layer from each funnel and discard.

Note: *Do not wait more than 5 minutes after the timer beeps before completing steps 14–16.*



14. Insert a cotton plug into the delivery tube of each separatory funnel. Slowly drain the toluene into respective sample cells labeled “blank” and “sample”. Stopper the sample cells.

Note: *Filtering the toluene through dry absorbent cotton will remove any water or suspended particles.*

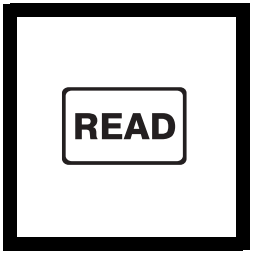
Note: *The developed color is stable but should be measured as soon as possible.*

15. Place the blank into the cell holder. Close the light shield. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. NA

DR/2010
Program No. 640
420 nm

DR/4000
Program No. 3300
420 nm



16. Place the sample in the cell holder. Read the mg/L selenium.

Sampling and Storage

Collect samples in clean glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 1.5 mL per liter). Preserved samples can be stored for up to six months at room temperature. Correct the test result for volume additions.

Distillation

Always perform this procedure under a fume hood! This distillation involves the use of a strong acid and oxidizer at high temperatures. To avoid personal injury, observe all laboratory safety precautions when operating the distillation apparatus.

a. Measure 500 mL of sample into a 1000-mL beaker.

b. Add 1 mL of Methyl Orange Indicator Solution to the beaker. Stir with a glass rod.

c. Using a dropper, add 0.1 N Hydrochloric Acid Standard Solution drop-wise until the solution turns pink. Add an additional 2 mL.

d. Pipet 5.0 mL Calcium Chloride Solution. Mix well.

e. Using a dropper, add 1-g/L Potassium Permanganate Standard Solution drop-wise until the solution is purple.

f. Place the beaker on a hot plate. Evaporate the solution to approximately 250 mL. Periodically add 1-g/L Potassium Permanganate Solution to keep the solution purple.

Note: *Any precipitate formed at this step is manganese dioxide and may be ignored.*

g. Cool the solution. While cooling, set up the distillation apparatus for the general purpose distillation as shown in the Hach Distillation Manual.

h. Pour the treated sample solution into the distillation flask. Add a stirring bar to the flask.

i. Pipet 5.0 mL of 0.1 N Sodium Hydroxide Standard Solution into the flask. Turn the stirrer power switch to ON. Set the stir control to 5.

j. Turn on the water and adjust so a constant flow is maintained through the condenser. Set the heat control to 10.

k. When only a few milliliters are left in the distillation flask, turn the power switch off. Discard the distillate in the erlenmeyer flask.

l. Perform this step under a hood. When the flask has cooled, add 50 mL of 19.2 N Sulfuric Acid Standard Solution to the flask. Add the contents of one Potassium Bromide Powder Pillow to the flask.

m. Fill a 250-mL beaker to the 75-mL mark with deionized water. Place it under the drip tube. Elevate the beaker with a laboratory jack so the tube extends below the level of the water.

n. Add 1.0 mL of 30% Hydrogen Peroxide Solution to the flask. Turn the stir control to 5 and the heat control to 10. Cap the distillation flask.

o. Heat the distillation flask until the yellow color is gone from the complete distillation apparatus, including the J-tube and condenser. Remove the beaker from under the drip tube.

p. Turn off the heater switch. When the J-tube and condenser have cooled, rinse them with deionized water. Add the washings to the 250-mL beaker. Total volume in the beaker should be approximately 100 mL.

q. Add the Phenol Solution drop-wise to the distilled sample to discharge the bromine color (a white precipitate of tribromophenol will form.)

r. Allow the precipitate to settle. Using a dropper, collect about 5 mL of the clear, colorless distillate and transfer it to a test tube.

s. Test the solution for completeness of precipitation by adding 2 drops of Phenol Solution. If the solution becomes cloudy or white precipitate forms, residual bromine is still present (proceed to next step). If no cloudiness occurs, the sample is ready for analysis.

t. Transfer the 5-mL aliquot back to the beaker and continue to add Phenol Solution until no turbidity is formed in subsequent 5-mL aliquots.

u. Transfer the sample into a 500-mL volumetric flask. Rinse the beaker with deionized water and add the rinse to the flask.

v. Dilute to volume with deionized water, stopper and mix well. The distillate is now ready for analysis.

Accuracy Check

Standard Additions Method

a. Use a TenSette Pipet to add 0.1, 0.2, and 0.3 mL of a Selenium Standard Solution, 100 mg/L, to three 100-mL samples. Mix well.

b. Analyze as described above. Each 0.1 mL addition of standard should increase the selenium concentration by 0.1 mg/L.

c. If these increases do not occur, an interference is likely.

Standard Solution Method

Prepare a 0.50-mg/L selenium standard solution as follows:

a. Use a TenSette Pipet or 1.00-mL volumetric pipet to add 1.00 mL of 100-mg/L Selenium Standard Solution to a 200-mL volumetric flask.

b. Dilute to the mark with deionized water. Transfer 100 mL of the standard into a 500-mL Erlenmeyer flask. Perform the test as described above.

Interferences

There are no positive inorganic interferences with this method.

Strong oxidizing agents such as iodine, bromine, or chlorine can react with the indicator to give low results.

Manganese and up to 2.5 mg/L ferric iron will not interfere.

Interferences will be eliminated by following the distillation procedure.

Summary of Method

An EDTA masking agent is added to the sample to remove interferences such as iron prior to the test. The addition of a sulfate buffer adjusts the sample to the optimum pH of 1 to 2. Under these conditions, diaminobenzidine reacts with all selenium present as selenite (Se^{4+}) to give a yellow-colored piazselenol complex which is extracted and the color intensity measured colorimetrically. Selenium present as Se^{2-} or Se^{6+} is not detected unless the sample is distilled.

REQUIRED REAGENTS

Description	Cat. No.
Selenium Reagent Set (45 Tests)	22442-00

Includes: (1) 452-49, (1) 7062-22, (2) 230-32, (1) 204-26, (1) 14470-17

	Quantity Required		
Description	**Per Test**	**Unit**	**Cat. No.**
Buffer Solution, sulfate type, pH 2.0	10 mL	500 mL	452-49
Diaminobenzidine, tetrahydrochloride	0.1 g	5 g	7062-22
Potassium Hydroxide Standard Solution, 12 N	4 mL	100 mL MDB	230-32
TitraVer Hardness Reagent	0.4 g	100 g	204-26
Toluene, ACS	60 mL	4 liters	14470-17
Water, deionized	100 mL	4 liters	272-56

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
Cotton Balls	1	100/pkg	2572-01
Cylinder, graduated, 100 mL	1	each	508-42
Cylinder, graduated, 50 mL	1	each	508-41
Dropper, 0.5 & 1.0 mL marks, glass	1	5/pkg	14197-05
Flask, erlenmeyer, 500 mL	2	each	505-49
Funnel, separatory, 250 mL	2	each	520-46
Pipet, volumetric, 5 mL	1	each	14515-37
Pipet Filler, safety bulb	1	each	14651-00
Ring, support, 83 mm (3 in.)	2	each	580-00
Sample Cells, matched pair, 1-in.	2	pair	20950-00
Spoon, measuring, 0.2 g	1	each	638-00
Spoon, measuring, 0.05 g	1	each	492-00
Squeezer, 0.025-1.00 mL plastic dropper	1	20/pkg	21247-20
Stand, support, 127 x 203 mm	1	each	563-00
Select one based on available voltage:			
Hot Plate, 4" diameter, 120 Vac	1	each	12067-01
Hot Plate, 4" diameter, 240 Vac	1	each	12067-02

OPTIONAL REAGENTS

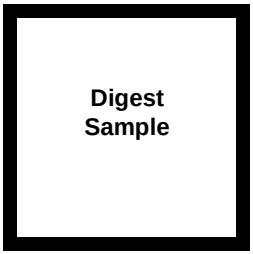
Acetone, ACS	500 mL	14429-49
Calcium Chloride Solution	1000 mL	428-53
Hydrochloric Acid Standard Solution, 0.1 N	1000 mL	14812-53
Hydrogen Peroxide, 30%	473 mL	144-11
Methyl Orange Indicator Solution, 0.1 N (0.05%)	500 mL	148-49
Nitric Acid, ACS	500 mL	152-49
Phenol Solution, 30 g/L	29 mL	2112-20
Potassium Bromide Powder Pillows	100/pkg	14819-99
Potassium Permanganate Standard Solution	100 mL	14164-42
Selenium Standard Solution, 1000 mg/L	100 mL	22407-42
Selenium Standard Solution, 100 mg/L, 2-mL ampules	20/pkg	12184-20
Sodium Hydroxide Standard Solution, 0.1 N	1000 mL	191-53
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	2450-32
Sulfuric Acid Standard Solution, 5.25 N	100 mL	2449-32
Sulfuric Acid Standard Solution, 19.2 N	500 mL	2038-49

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Beaker, 250 mL	each	500-46
Beaker, 1000 mL	each	500-53
Bottle, wash, 500 mL	each	620-11
Clippers, for opening powder pillows	each	968-00
Cylinder, graduated, 500 mL	each	508-49
Distillation apparatus for general purpose	each	22653-00
Distillation apparatus heater, 115 V	each	22744-00
Distillation apparatus heater, 230 V	each	22744-02
Dropper, 0.5 and 1 mL marks	6/pkg	23185-06
Flask. volumetric, Class A, 500 mL	each	14574-49
Jack, laboratory	each	22743-00
pH Meter, *sens**ion***™*1*, portable	each	51700-10
Pipet, serological, 10 mL	each	532-38
Pipet, TenSette,® 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Pipet, volumetric, Class A, 1.00 mL	each	14515-35
PourRite™ Ampule Breaker	each	24846-00
Rod, stirring, glass	3/pkg	1770-01
Stoppers, for cells, hollow No. 1	6/pkg	14480-00

SILVER

Colorimetric Method
Range: Liquids- 0.08–6000 mg/L; Solids- 6–60000 mg/kg

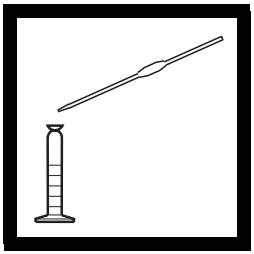


1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

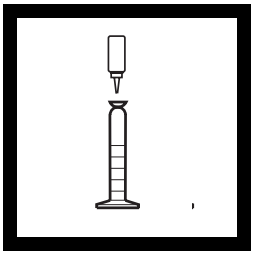
Note: *If samples cannot be analyzed immediately, see Sampling and Storage following these steps.*

Note: *The Pour/Flow Thru cell cannot be used with this procedure.*

Note: *To screen for very low levels (0-50 ppb) of silver, use Hach's RapidSilver™ Test Kit (Cat. No. 26745-00).*

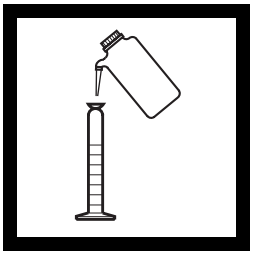


2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 50-mL mixing cylinder.

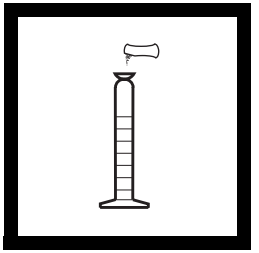


3. Add one drop of Thymolphthalein Indicator Solution and one drop of Phenolphthalein Indicator Solution to the cylinder. Use sodium hydroxide to adjust the solution to a pH of 9-10. The solution should be pink.

Note: *A purple color indicates a pH greater than 10. To readjust the pH, add a drop of sulfuric acid and one drop of each of the indicators and repeat step 3.*

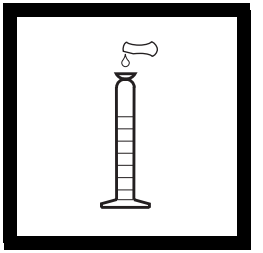


4. Dilute to the 50 mL mark with deionized water.



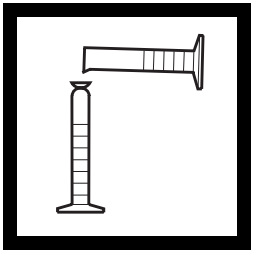
5. Add the contents of one Silver 1 Powder Pillow to a clean, dry 50-mL graduated mixing cylinder.

Note: *If the Silver 1 Powder becomes wet at this point, the powder will not dissolve completely, which will inhibit color development.*

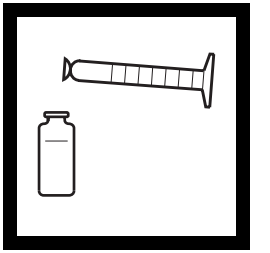


6. Add the contents of one Silver 2 Reagent Solution Pillow to the cylinder. Swirl to completely wet the powder.

Note: *If clumps of dry powder are present when the sample is poured in, the powder will not dissolve completely. This will inhibit color formation.*



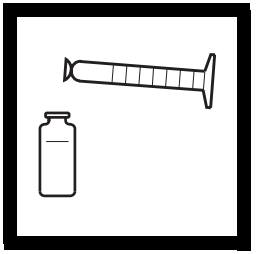
7. Pour the sample from step 2 into the cylinder containing the reagent powder. Stopper. Invert repeatedly for one minute.



8. Pour 25 mL of the mixture into a sample cell (the blank). Add the contents of one Thiosulfate Powder Pillow to the sample cell. Swirl for 30 seconds to mix.

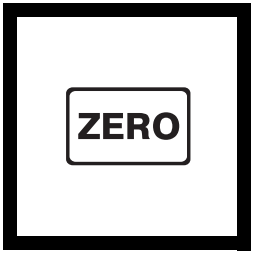
Note: *It is important to generate a blank for each sample.*

9. Begin a two-minute reaction period.



10. Pour the portion remaining in the cylinder into a second sample cell (the prepared sample).

When the timer beeps, place the blank in the cell holder. Close the light shield.

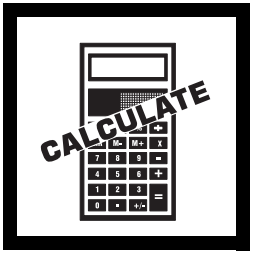


11. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. NA

DR/2010
Program No. 660
560 nm

DR/4000
Program No. 3400
560 nm



12. Place the sample in the cell holder. Read the mg/L silver.

Use the equation below the Silver Sample and Analysis Volume Tables to calculate the true silver concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Ag Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.08-3.7	40.0	20.0	50 mL
0.3-15	20.0	10.0	50 mL
1.2-60	10.0	5.00	50 mL
12-600	5.00	1.00	50 mL
120-6000	1.00	0.50	50 mL

Solids

Expected Ag Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
6-300	0.500	20.0	50 mL
15-750	0.400	10.0	50 mL
40-2000	0.300	5.00	50 mL
300-15000	0.200	1.00	50 mL
1200-60000	0.100	0.50	50 mL

Calculation For Final Concentration:

$$\frac{A \times 5000}{B \times C} = \text{mg/kg or mg/L Total Ag}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-cleaned plastic or glass bottles. Using pH paper, adjust the pH to 2 or less with nitric acid (about 2 mL/liter). Store preserved samples at room temperature for up to 6 months. Before analysis, adjust the pH to 9–10 with 5.0 N sodium hydroxide. Do not use a pH meter because of silver contamination from the electrode. Correct for volume additions.

Accuracy Check

Standard Additions Method

a. Add 5.0 mL of 1000-mg/L Silver Solution to a 100-mL volumetric flask. Dilute to volume with deionized water. Mix well. This is a 50-mg/L silver standard solution.

b. Use a TenSette Pipet to add 0.1, 0.2 and 0.3 mL of this standard solution to three 50-mL samples (or sample portions diluted to 50 mL). Mix well.

c. Analyze as described above. Each 0.1 mL addition of standard should increase the silver concentration by 0.1 mg/L.

d. If these increases do not occur, an interference is likely.

Standard Solution Method

Prepare a 0.50-mg/L silver standard solution as follows:

a. Use a Class A 0.50-mL volumetric pipet to add 0.50 mL of 1000-mg/L Silver Standard Solution to a 1000-mL volumetric flask.

b. Dilute to the mark with deionized water and mix. Prepare this solution daily. Perform the silver test as described. Results should be between 0.45 and 0.55 mg/L Ag.

Interferences

Interference studies were conducted by preparing a known silver solution (about 0.4 mg/L) and the potential interfering ion. The ion was said to interfere when the silver concentration changed by ±10%.

Negative Interference:		
	Aluminum	30 mg/L
	Ammonia	750 mg/L
	Cadmium	15 mg/L
	Chloride	19 mg/L*
	$Chromium^{6+}$	90 mg/L
	Copper	7 mg/L
	Iron	30 mg/L
	Lead	13 mg/L
	Manganese	19 mg/L
	Nickel	19 mg/L
	Zinc	70 mg/L
Positive Interference:		
	Calcium	600 mg/L
	Magnesium	2000 mg/L
	Mercury	2 mg/L

* Monitor chloride with Hach Water Quality Test Strips for Low Range Chloride (Cat. No. 27449-40).

Summary of Method

Silver ions in basic solution react with cadion 2B to form a green, brown or red-purple complex. Sodium thiosulfate acts as a decolorizing agent for the blank. The Silver 1 and Silver 2 reagents contain the buffer, indicator and masking agents. Organic extractions are not necessary and this method does not have as many interferences as the traditional dithizone method. It may also be used for electroplating and silver strike solutions.

REQUIRED REAGENTS

Description	Cat. No.
Silver Reagent Set (50 tests)	22966-00

Includes: (1) 22935-66, (1) 22936-66, (1) 22937-66

Description	Quantity Required Per Test	Units	Cat. No.
Silver 1 Powder Pillow	1 pillow	50/pkg	22935-66
Silver 2 Solution Pillow	1 pillow	50/pkg	22936-66
Sodium Thiosulfate Powder Pillow	1 pillow	50/pkg	22937-66

REQUIRED APPARATUS

Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 50 mL	1	each	21179-41
Cylinder, graduated, mixing, 50 mL	1	each	1896-41
Phenolphthalein Indicator Solution, 1 g/L	1 drop	15 mL SCDB	1897-36
Sulfuric Acid, ACS	varies	2.5 L	979-09
Thymolphthalein Indicator Solution, 1 g/L	1 drop	15 mL SCDB	21853-36
Water, deionized	50 mL	4 L	272-56

Select one or more based on sample amount and analysis volume:

Pipet, volumetric, Class A, 20.0 mL	1	each	14515-20
Pipet, volumetric, Class A, 10.0 mL	1	each	14515-38
Pipet, volumetric, Class A, 5.0 mL	1	each	14515-37
Pipet, volumetric, Class A, 1.0 mL	1	each	14515-35
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

OPTIONAL REAGENTS

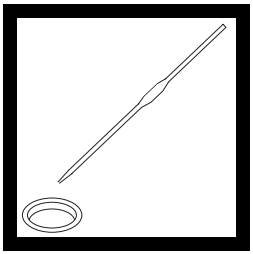
RapidSilver™ Test Kit		each	26745-00
Silver Standard Solution, 1000 mg/L Ag		100 mL	14613-42
Sodium Hydroxide Solution, 1.0 N		100 mL MDB	1045-32
Sodium Hydroxide Solution, 5.0 N		100 mL MDB	2450-32
Sodium Hydroxide, 50%		500 mL	2180-49
Water Quality Test Strips, low range chloride		40/pkg	27449-40

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Boiling Chips, silicon carbide	500 g	20557-34
Flask, volumetric, Class A, 100 mL	each	14574-42
Flask, volumetric, Class A, 1000 mL	each	14574-53
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipet, serological, 10.0 mL	each	532-38
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet, TenSette®, 0.1 to 10.0 mL	each	19700-10
Pipet Tips, for 19700-01 Pipet	50/pkg	21856-96
Pipet Tips, for 19700-10 Pipet	50/pkg	21997-96
Pipet, volumetric, Class A, 0.50 mL	each	14515-34
Pipet Filler, safety bulb	each	14651-00
Safety Goggles	each	18421-00
Safety Shield, for Digesdahl	each	50040-00

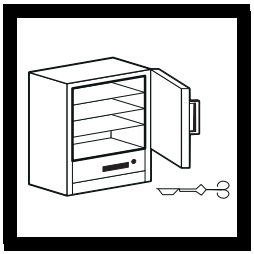
SOLIDS, TOTAL

Gravimetric Method



1. Mix sample. Add 50 mL to a preweighed (to nearest 0.1 mg) aluminum dish.

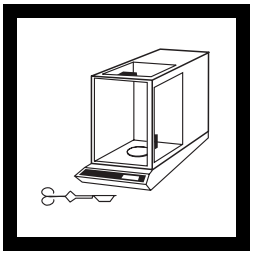
Note: *If volatile solids are to be measured, ignite the aluminum dishes for 1 hour at 550 °C before use.*



2. Evaporate sample in an oven at 103-105 °C.

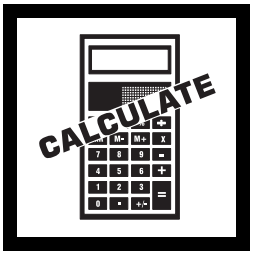
Note: *Drying will take approximately 6 hours. The oven should be preheated to ensure adequate drying. Highly mineralized water may require prolonged drying.*

3. Take dish out of oven and allow to cool to room temperature in a desiccator.



4. Weigh the dish to the nearest 0.1 mg using an analytical balance.

Note: *Repeat drying (approximately 15 minutes) at 103-105 °C until results do not differ by more than 0.4 mg. Successive weighings that are identical for some wastewater samples are unlikely due to slow organic volatilization.*



5. Calculations:

$$\text{mg/L Total Solids} = \frac{(A - B) \times 1000}{\text{Sample Volume in mL}}$$

Where:

A = Weight (mg) of sample + dish

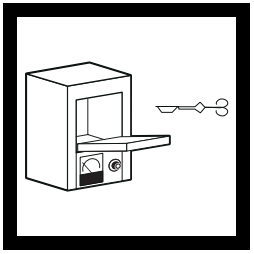
B = Weight (mg) of dish

REQUIRED APPARATUS

Description	Unit	Cat. No.
Balance, analytical	each	26103-00
Cylinder, 50 mL	each	508-41
Desiccant, Indicating Drierite	454 g	20887-01
Desiccator, without stopcock	each	14285-00
Desiccator Plate, ceramic	each	14284-00
Dish, aluminum (63 x 17.5 mm)	100/pkg	21640-00
Furnace, muffle	each	14296-00
Oven, laboratory, 120V, 60 Hz	each	14289-00
Pipet, serological, 25 mL	each	2066-40
Tongs	each	569-00

SOLIDS, TOTAL VOLATILE AND FIXED

Gravimetric Method

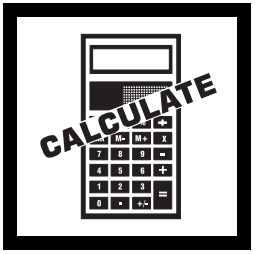


1. Transfer the aluminum dish from *step 4* of the Total Solids Method (8271) into muffle furnace at 550 °C for 30 minutes.

Note: *The furnace should be preheated to 550 °C to ensure adequate ignition.*

2. Take dish out of furnace with tongs and cool to room temperature in a desiccator.
Weigh dish to the nearest 0.1 mg using an analytical balance.

Note: *Repeat ignition until two successive sample weighings (Example: A – B as in* step 3*) do not differ by more than 4% or 0.5 mg, whichever is less.*



3. Calculations: Loss of weight is total volatile solids. Weighed residue is total fixed solids.

$$\text{mg/L Volatile Solids} = \frac{(A - B) \times 1000}{\text{sample volume in mL}}$$

Where:

A = Weight (mg) of solids + dish before ignition

B = Weight (mg) of solids + dish after ignition

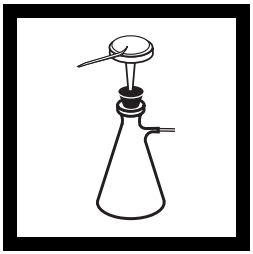
C = Weight (mg) of dish

REQUIRED APPARATUS

Description	Unit	Cat. No.
Balance, analytical	each	26103-00
Desiccant, Drierite indicator	454 g	20887-01
Desiccator, without stopcock	each	14285-00
Desiccator Plate, ceramic	each	14284-00
Dishes, aluminum (63 x 17.5 mm)	100/pkg	21640-00
Furnace, muffle	each	14296-00
Oven, laboratory, 120V, 60 Hz	each	14289-00
Tongs	each	569-00

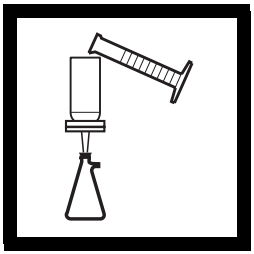
SOLIDS, SUSPENDED, TOTAL & VOLATILE AND TOTAL DISSOLVED SOLIDS

Total Suspended (Nonfilterable) Solids Gravimetric Method* (USEPA Accepted))

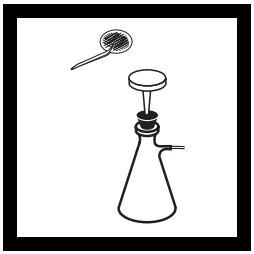


1. Place a 47-mm glass fiber filter disc in the filter holder with the wrinkled surface up.

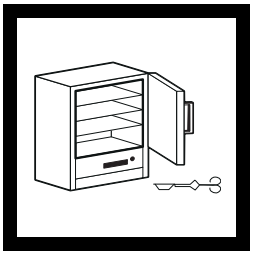
Note: *A glass filter must be used. ALWAYS use tweezers to handle filter discs. Fingers add moisture which will cause a weighing error.*



2. Place the filter holder assembly in the filtering flask and add 100 mL of deionized water. Apply vacuum to the flask until all the water is drawn through the filter.



3. Remove the disc from the filter holder and transfer to a watch glass.



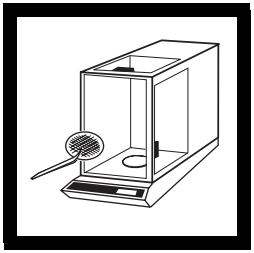
4. Place in a drying oven at 103–105 °C for one hour.

Note: *Preheat oven to 103° C to ensure adequate drying of the filter disc.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater* and *Methods for Chemical Analysis of Water and Wastewater* (USEPA).

5. Remove the disc with watch glass from the oven and place in a desiccator. Allow to cool to room temperature.

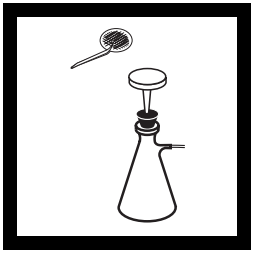
Note: *Use metal tongs to transfer the watch glass and filter disc from the oven directly into the desiccator. Cover immediately. Allow the vessel to cool slightly before sealing the desiccator as pressure from the heated air inside the desiccator can force the cover off.*



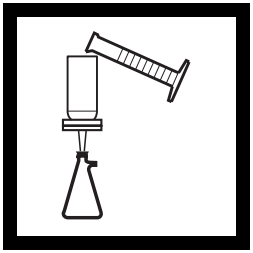
6. Remove the disc from the desiccator and weigh to the nearest 0.1 mg using an analytical balance.

Note: *Remove the watch glass and disc from the desiccator as a unit and place beside the analytical balance. Use plastic tweezers to remove the disc from the watch glass and to transfer the disc to and from the weighing pan of the balance.*

Note: *Repeat drying procedure until a constant weight is obtained or until weight loss is <0.5 mg.*

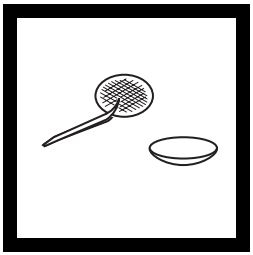


7. Again place the disc in the filter holder/flask assembly with the wrinkled surface upward. Wet the disc with deionized water to ensure adhesion to the holder.



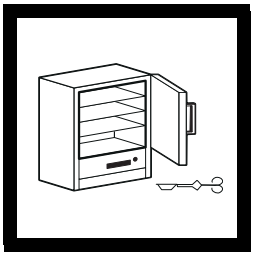
8. Filter 100 mL (or more if solids content is low) of well-mixed, representative water sample by applying vacuum to the flask. Follow with three separate 10-mL washings of deionized water.

Note: *For greatest accuracy, as much sample as possible should be filtered. However, using a sample containing more than 15 mg of solids will result in premature plugging of the filter. The exact volume of the water sample may have to be adjusted (increased or decreased) to achieve this optimum condition. Several completed tests will show whether any adjustment is necessary.*



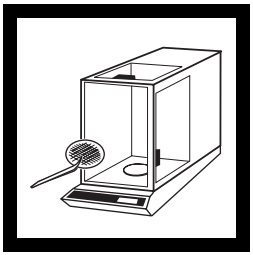
9. Slowly release the vacuum from the filtering system and gently remove the filter disc from the holder. Place the disc on a watch glass. Inspect the filtrate (filtered water in flask) to ensure that proper trapping of solids was accomplished on the disc.

Note: *Be sure to remove any residue adhering to the sides or bottom lip of the filter holder. A rubber policeman on the end of a stirring rod is very helpful in scraping this residue loose, and small amounts of deionized water will help wash the residue down onto the filter disc.*



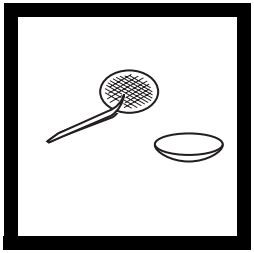
10. Again place the watch glass and filter in a drying oven at 103–105 °C for one hour.

11. Remove the watch glass and filter from the oven, and carefully place in a desiccator. Cool to room temperature.



12. Carefully remove the disc from the desiccator and weigh to the nearest 0.1 mg using an analytical balance.

Note: *Take extreme care when removing the lid of the desiccator to not disturb the dried suspended matter on the disc. Remove the watch glass and disc from the desiccator as a unit and place beside the analytical balance. Use plastic tweezers to transfer the disc to and from the weighing pan of the balance.*



13. Return the disc to the watch glass if the mg/L Volatile Nonfilterable Residue is to be determined. If not, discard the disc.

Note: *If Volatile Nonfilterable Residue also is to be determined, take care not to lose any portion of the suspended matter on the disc.*

Note: *Repeat the drying procedure until a constant weight is obtained or until the weight loss is <0.5 mg.*

$$\frac{\text{A - B}}{\text{Sample Volume in Liters}} = \text{mg/L TSS}$$

14. Calculate Total Suspended Solids (TSS):

$$\frac{\text{A} - \text{B}}{\text{Sample Volume in Liters}} = \text{mg/L TSS}$$

Where:

A = Weight (mg) of disc with residue

B = Weight (mg) of disc

Example:

A = 95.5 mg

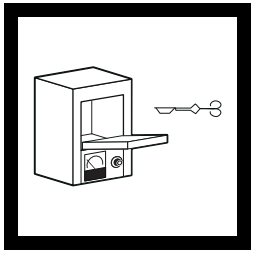
B = 81.5 mg

Sample volume = 0.1 L

$$\frac{(95.5\text{mg}) - (81.5\text{mg})}{0.1\text{Liters}} = \text{TSS}$$

$$\frac{14.0}{0.1} = 140 \text{ mg/L TSS}$$

Volatile Suspended (Nonfilterable) Solids — Method 8164

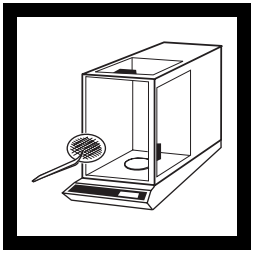


1. Place the watch glass and filter disc from the Total Suspended Solids procedure (*step 13*) in the muffle furnace and ignite at 550 °C for 60 minutes.

Note: *The muffle furnace may be partially preheated before inserting the watch glass. However, placing the watch glass in a 550°C furnace could cause it to shatter. The temperature should be brought up to 550°C after placing the filter and watch glass in the oven and held at that temperature for about 60 minutes. Use tongs to transfer the watch glass and filter disc into the oven as a unit.*

2. Remove the watch glass and filter from the furnace and carefully transfer into the desiccator. Allow to cool to room temperature.

Note: *Use metal tongs to transfer the watch glass and filter disc from the oven or furnace directly into the desiccator. Cover immediately. Allow the vessel to cool slightly before sealing the desiccator as pressure from the heated air inside the desiccator can force the cover off.*



3. Carefully remove the filter disc from the desiccator and weigh to the nearest 0.1 mg using an analytical balance.

Note: *Take extreme care when removing the lid of the desiccator to not disturb the dried suspended matter on the disc. Remove the watch glass from the desiccator as a unit and place beside the analytical balance. Use plastic tweezers to transfer the disc to and from the weighing pan of the balance. Discard the filter disc.*

4. Calculate Volatile Suspended Solids (VSS).

$$\frac{A - B}{\text{Sample Volume in Liters}} = \text{mg/L VSS}$$

Where:

A = Weight (mg) of residue and disc before ignition.

B = Weight (mg) of residue and disc after ignition.

Example:

A = 95.5 mg

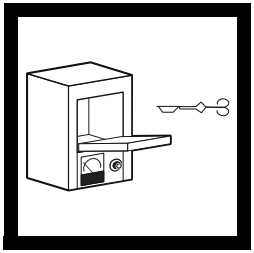
B = 91.2 mg

Sample Volume = 0.1 L

$$\frac{(95.5\text{ mg}) - (91.2\text{ mg})}{0.1\text{ Liters}} = \text{mg/L VSS}$$

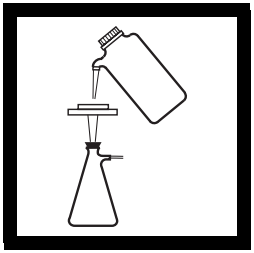
$$\frac{4.3}{0.1} = 43\text{ mg/L VSS}$$

Total Dissolved (Filterable) Solids — Method 8163

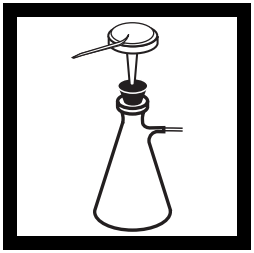


1. Heat a cleansed evaporating dish in a muffle furnace at 550 °C for one hour. Transfer directly into a desiccator and store until needed.

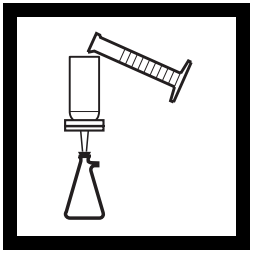
Note: *Use metal tongs to transfer the evaporating dish from the oven or furnace directly into the desiccator. Cover immediately. Allow the vessel to cool slightly before sealing the desiccator as pressure from the heated air inside the desiccator can force the cover off.*



2. Assemble the filter holder/flask assembly, using a clean filter flask. All residue should be removed from the flask by cleansing thoroughly with a dilute solution of ammonium hydroxide, followed by rinsing with distilled water.



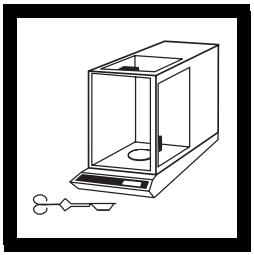
3. Place a 47-mm filter disc in the filter holder with the wrinkled surface upward and, with vacuum applied to the flask, wash the filter with three separate 20-mL volumes of distilled water. Remove all traces of water by continuing vacuum for two to three minutes after the water has passed through the filter. Disconnect the vacuum, and discard these washings from the flask.



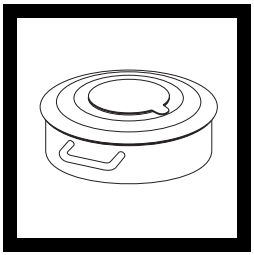
4. Reconnect vacuum to the filter holder/flask assembly and, using a clean 100-mL graduated cylinder, filter 100 mL (or more if solids content is low) of a well-mixed representative water sample.

Note: *For greatest accuracy, as much sample as possible should be filtered. However, using a sample containing more than 15 mg of solids will result in premature plugging of the filter. The exact volume of the water sample may have to be adjusted (increased or decreased) to achieve this optimum condition. Several completed tests will show whether any adjustment is necessary.*

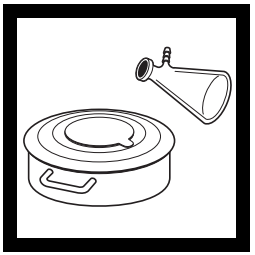
5. Apply vacuum for two to three minutes after the sample has passed through the filter; then disconnect the vacuum.



6. Using tongs, transfer the evaporating dish from the desiccator to the balance. Weigh to the nearest 0.1 mg and record this weight.

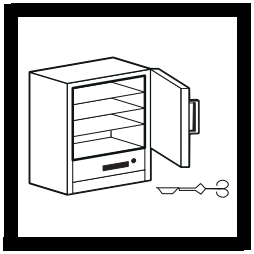


7. Place the steam bath on the hot plate, add water and transfer the evaporating dish from the balance to the steam bath.

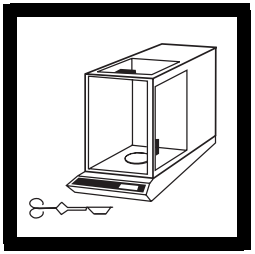


8. Pour the 100-mL filtrate sample from the filter flask into the evaporating dish and evaporate to dryness.

Note: *Evaporation of 100 mL of filtrate may take as long as four hours. Be sure to check the reservoir of the water bath occasionally and add more water when needed.*



9. Using tongs, transfer the evaporating dish residue to a drying oven and dry at 180 °C for one hour. Transfer to a desiccator and cool.



10. Weigh the evaporating dish to the nearest 0.1 mg on an analytical balance and record this weight.

Repeat Steps 9 and 10

11. Repeat *step 9* and *step 10* until a constant weight is obtained, or the change is <4% or 0.5 mg.

$$\frac{\text{A - B}}{\text{Sample Volume in Liters}} = \text{mg/L TDS}$$

12. Calculate Total Dissolved Solids (TDS):

$$\frac{A - B}{\text{Sample Volume in Liters}} = \text{mg/L TDS}$$

Where:

A = Weight (mg) of residue and dish after drying.

B = Weight (mg) of dish.

Sample Volume = 0.1 L

Example:

A = 20187.3 mg

B = 20140.1 mg

$$\frac{20187.3 - 20140.1}{0.1} = \text{mg/L TDS}$$

$$\frac{47.2}{0.1} = 472 \text{ mg/L TDS}$$

Sampling and Storage

Collect samples in clean plastic or glass bottles. Samples should be analyzed as soon as possible after collection but can be stored up to seven days by cooling to 4 °C (39 °F).

REQUIRED REAGENTS AND APPARATUS

- For Total Suspended Solids

Description	Quantity Required Per Test	Unit	Cat. No.
Aspirator, vacuum	1	each	2131-00
Balance, analytical	1	each	26103-00
Bottle, wash, 500 mL	1	each	620-11
Cylinder, graduated, 100 mL	1	each	508-42
Desiccator plate, 230 mm	1	each	14284-00
Desiccator, 250 mm, without stopcock	1	each	14285-00
Drierite, with indicator	varies	454 g	20887-01
Filter disc, glass fiber, 47 mm	1	100/pkg	2530-00
Filter Holder, magnetic	1	each	13529-00
Flask, filtering, 1000 mL	1	each	546-53
Oven, laboratory, ambient to 200°C, ±1 °C	1	each	14289-00
Stopper, rubber, one-hole, No. 8	1	6/pkg	2119-08
Tongs	1	each	569-00
Tubing, rubber, 7.9 x 2.4 mm	1	3.6 m	560-19
Tweezers, plastic	1	each	14282-00
Watch Glass, 100 mm	1	each	578-70
Water, deionized	varies	4 L	272-56

REQUIRED APPARATUS- For Volatile Suspended Solids

For this test, use the Required Reagents and Apparatus listed above, plus the following:

Furnace, muffle type, 120 V, 50/60 Hz	1	each	14296-00
Furnace, muffle type, 240 V, 50/60 Hz	1	each	14296-24

REQUIRED APPARATUS- For Total Dissolved Solids

For this test, use the Required Reagents and Apparatus listed above, plus the following:

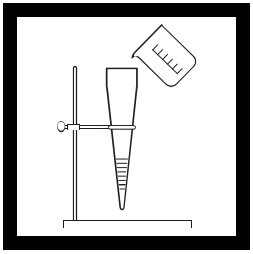
Cylinder, graduated, 100 mL	1	each	508-42
Evaporating dish, porcelain, 120 mL, 90mm, size 2	1	each	525-61
Hot Plate, Thermolyne CIMAREC, 120 V, 50/60 Hz	1	each	23441-00
Hot plate, Thermolyne CIMAREC, 240 V, 50/60 Hz	1	each	23441-02
Steam Bath, 8 in. diameter, copper w/concentric rings	1	each	23479-00

OPTIONAL REAGENTS AND APPARATUS

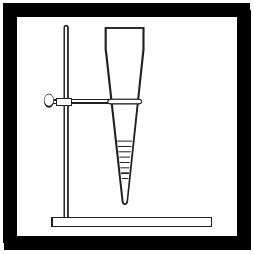
Ammonium Hydroxide, approx. 58% ACS		500 mL	106-49
Brush, cylinder, size 2		each	687-00
Pump, vacuum, hand-operated		each	14283-00
Pump, vacuum/pressure, portable		each	14697-00
Rubber policeman for 1/8-in. rod		each	14309-00
Stirring rod, glass		3/pkg	1770-01

SOLIDS, SETTLEABLE MATTER

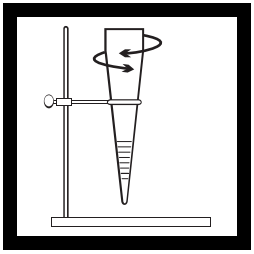
Direct Measurement Method (USEPA Approved)



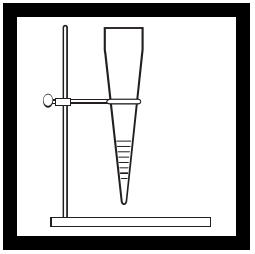
1. Fill an Imhoff cone to the 1-liter mark with a thoroughly mixed sample.



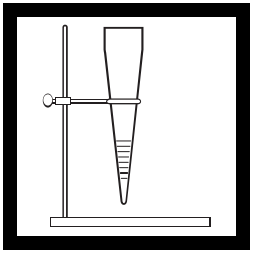
2. Let the sample sit undisturbed for 45 minutes.



3. Spin the cone forward and backward several times to dislodge materials on the inclined side of the cone.



4. Let the sample sit undisturbed for 15 minutes.



5. Find the mark at the top of the solids layer on the graduated scale of the Imhoff cone. This is the mL/L of settleable matter.

Sampling and Storage

Collect samples in a clean plastic or glass bottle. If sample cannot be analyzed immediately, store at 4 °C to minimize microbial decomposition. Analyze within 24 hours.

Summary of Method

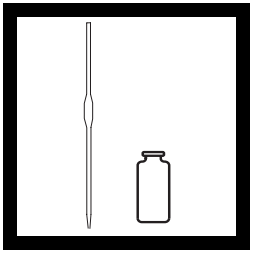
The amount of settleable matter in sewage treatment plant influent and effluent gives an empirical estimate of the type and extent of treatment required and the general quality of the water being discharged.

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
Imhoff Cone	1	each	2067-00
Imhoff Cone Support	1	each	572-00
Imhoff Cone Brush	1	each	688-00

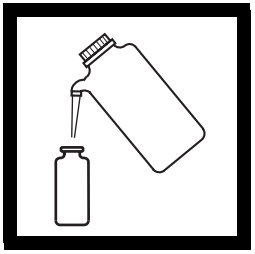
SULFIDE

Methylene Blue Method* (USEPA approved for reporting wastewater analysis)
Range: 0 to 0.600 mg/L S^{2-}

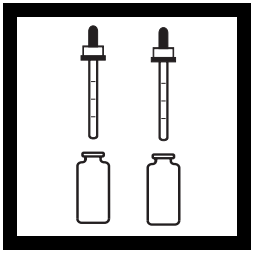


1. Pipet 25 mL of sample into a clean sample cell.

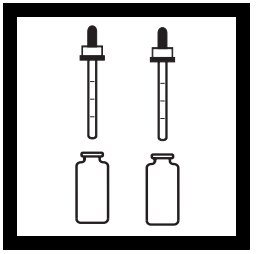
Note: *Samples must be analyzed immediately and cannot be preserved for later analysis. Avoid excessive agitation. Use a pipet to minimize loss of sulfide in the sample.*



2. Fill a second sample cell with 25 mL of deionized water (the blank).



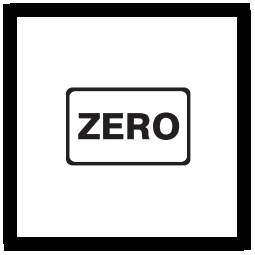
3. Add 1.0 mL of Sulfide 1 Reagent to each cell. Swirl to mix.



4. Add 1.0 mL of Sulfide 2 Reagent to each cell. Immediately swirl to mix.

Note: *A pink color will develop, then the solution will turn blue if sulfide is present.*

5. Begin a 5-minute reaction period.



6. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 93

DR/2010
Program No. 690
665 nm

DR/4000
Program No. 3500
665 nm

7. Immediately place the prepared sample into the cell holder. Close the light shield. Read the mg/L sulfide.

* Procedure is equivalent to USEPA method 376.2 of Standard Method 4500-S^{2-} D for wastewater.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation or prolonged exposure to air. Analyze samples immediately.

Interferences

For turbid samples, prepare a sulfide-free blank as follows. Use it in place of the deionized water blank in the procedure.

a. Measure 25 mL of sample into a 50-mL Erlenmeyer flask.

b. Add Bromine Water dropwise with constant swirling until a permanent yellow color just appears.

c. Add Phenol Solution dropwise until the yellow color just disappears. Use this solution in *step 4* in place of deionized water.

Strong reducing substances such as sulfite, thiosulfate and hydrosulfite interfere by reducing the blue color or preventing its development. High concentrations of sulfide may inhibit full color development and require sample dilution. Some sulfide loss may occur when the sample is diluted.

Soluble Sulfides

Determine soluble sulfides by centrifuging the sample in completely filled, capped tubes and analyzing the supernatant. Insoluble sulfides are then estimated by subtracting the soluble sulfide concentration from the total sulfide result.

Summary of Method

Hydrogen sulfide and acid-soluble metal sulfides react with N, N-dimethyl-p-phenylenediamine oxalate to form methylene blue. The intensity of the blue color is proportional to the sulfide concentration.

High sulfide levels in oil field waters may be determined by proper sample dilution.

REQUIRED REAGENTS

	Cat. No.
Sulfide Reagent Set (100 tests)	22445-00
Includes: (2) 1816-32, (2) 1817-32	

Description	Quantity Required Per Test	Units	Cat. No.
Sulfide 1 Reagent	2 mL	100 mL MDB	1816-32
Sulfide 2 Reagent	2 mL	100 mL MDB	1817-32
Water, deionized	25 mL	4 L	272-56

REQUIRED APPARATUS

Cylinder, graduated, 25 mL	1	each	508-40
Pipet, volumetric, Class A, 25.0 mL	1	each	14515-40
Pipet Filler, safety bulb	1	each	14651-00

OPTIONAL REAGENTS

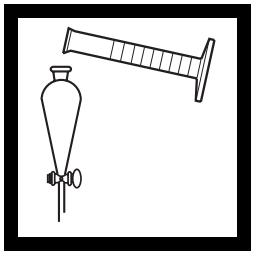
Bromine Water, 30 g/L	29 mL	2211-20
Phenol Solution, 30 g/L	29 mL	2112-20

OPTIONAL APPARATUS

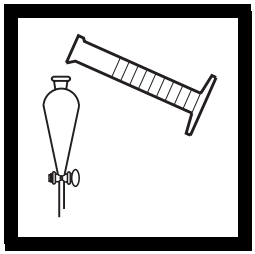
Bottle, Wash, 250 mL	each	620-31
Dropper, for 1 oz. (29 mL) bottle	each	2258-00
Flask, Erlenmeyer, 50 mL	each	505-41
Pipet, TenSette®, 1.0 to 10.0 mL	each	19700-10
Pipet, tips for TenSette® Pipet 19700-10	50/pkg	21997-96

SURFACTANTS, ANIONIC (also called Detergents)

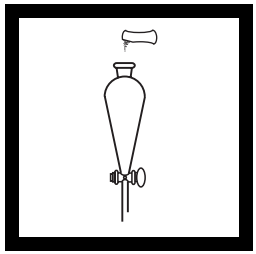
Crystal Violet Method*
Range: 0–0.275 mg/L



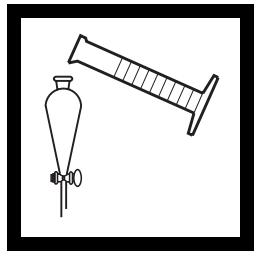
1. Fill a clean 500-mL graduated cylinder to the 300-mL mark with sample. Pour the sample into a clean 500-mL separatory funnel.



2. Add 10 mL of Sulfate Buffer Solution. Stopper the funnel. Shake the funnel for five seconds.



3. Add the contents of one Detergent Reagent Powder Pillow to the funnel. Stopper the funnel and shake to dissolve the powder.

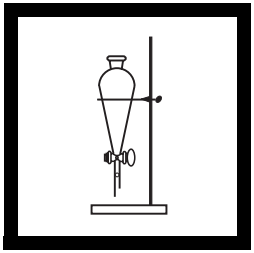


4. Add 30 mL of benzene to the funnel. Stopper the funnel and shake gently for one minute.

Note: *Spilled reagent will affect test accuracy and is hazardous to the skin and other materials.*

Note: *Use benzene only in a well-ventilated area.*

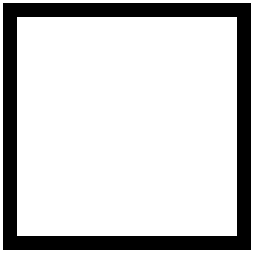
* *Analytical Chemistry,* 38, 791 (1966).



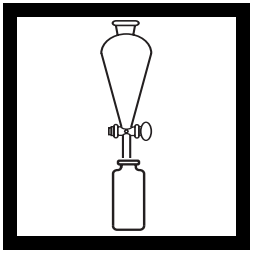
5. Place the separatory funnel in a support stand.

6. Begin a 30-minute reaction period.

Note: *Excessive agitation may cause an emulsion to form, requiring a longer time for phase separation. For these samples, remove most of the water layer, then gently agitate the funnel with a clean inert object in the funnel such as a Teflon-coated magnetic stirring bar.*

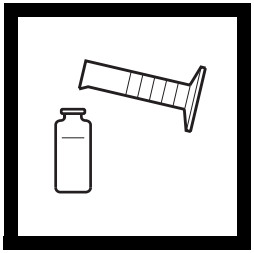


7. After the timer beeps, remove the stopper and drain the bottom water layer. Discard this layer.

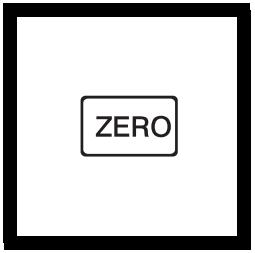


8. Drain the top benzene layer into a clean 25-mL sample cell (the prepared sample).

Note: *The benzene layer cannot be filtered before color measurement. Filtration removes the blue color.*



9. Fill another sample cell to the 25-mL mark with pure benzene (the blank).

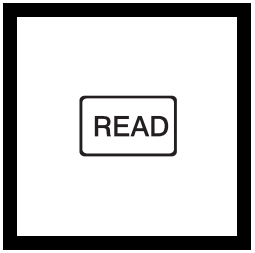


10. Zero the instrument with the blank, using the settings below.

DR/800s
Program No. 26

DR/2010
Program No. 710
605 nm

DR/4000
Program No.1850
605 nm



11. Place the prepared sample into the cell holder. Close the light shield. Read the mg/L anionic surfactants.

Note: *Acetone may be used to clean benzene from glassware.*

Note: *The prepared sample and blank must be disposed of according to current Federal, State, and local regulations for benzene.*

Sampling and Storage

Collect samples in clean plastic or glass bottles. Analyze samples as soon as possible, but they may be stored at least 24 hours by cooling to 4 °C (39 °F). Warm to room temperature before testing.

Accuracy Check

Standard Additions Method

a. Snap the neck off a Detergent Voluette Ampule Standard solution, 60 mg/L as LAS.

b. Using the TenSette Pipet, add 0.1, 0.2, and 0.3 mL of standard to three 300-mL samples. Mix thoroughly.

c. Analyze each as described above. The anionic surfactants reading should increase 0.02 mg/L for each 0.1 mL of standard added.

d. If these increases do not occur, an interference is likely.

Interferences

Perchlorate and periodate ions will interfere. High amounts of chloride, such as those levels found in brines and seawater, will cause low results.

Summary of Method

Detergents, ABS (alkyl benzene sulfonate) or LAS (linear alkylate sulfonate) are determined by association with crystal violet dye and extraction of the ion-pair complex into benzene.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Benzene, ACS	55 mL	500 mL	14440-49
Buffer Solution, sulfate type	10 mL	500 mL	452-49
Detergent Reagent Powder Pillow	1 pillow	25/pkg	1008-68

REQUIRED APPARATUS

Clippers, for opening powder pillows	1	each	968-00
Cylinder, graduated, 25 mL	1	each	508-40
Cylinder, graduated, 50 mL	1	each	508-41
Cylinder, graduated, 500 mL	1	each	508-49
Funnel, separatory, 500 mL	1	each	520-49
Ring, support, 4-inch	1	each	580-01
Stand, support, 127 x 203 mm	1	each	563-00

OPTIONAL REAGENTS

Acetone, ACS	500 mL	14429-49
Detergent Standard Solution, Voluette™ ampule, 60 mg/L as LAS, 10 mL	16/pkg	14271-10

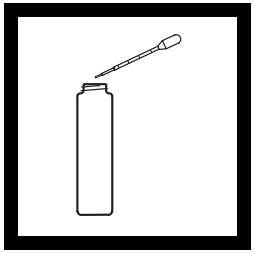
OPTIONAL APPARATUS

Ampule Breaker Kit	each	21968-00
Pipet, TenSette®, 0.1 to 1.0 mL	each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet	50/pkg	21856-96
Stir Bar, 9 mm	each	20953-48
Thermometer, -10 to 110 °C	each	1877-01

TOXICITY*

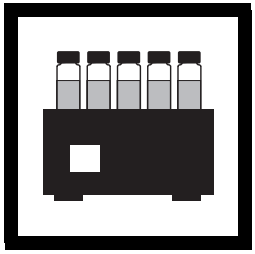
ToxTrak™ Colorimetric Method**
Range: 0–100% inhibition

Inoculum Development

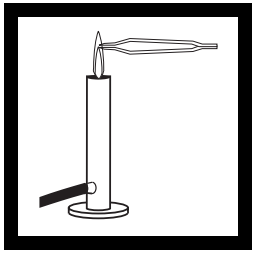


Using Indigenous Biomass

1. Using one of the dropper pipets provided, add 1.0 mL of source culture to a Tryptic Soy Broth Tube.

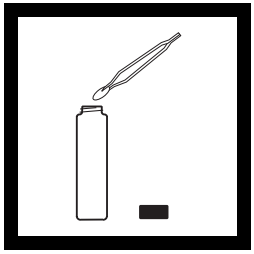


2. Incubate until the vial contents are visibly turbid (turbidity indicates bacterial growth).

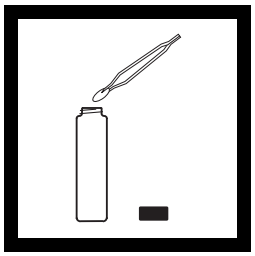


Using Bactrol Disks

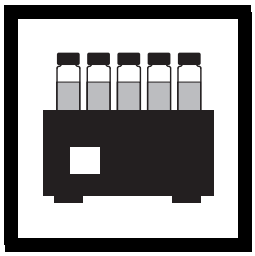
1. Flame sterilize forceps by dipping into alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool.



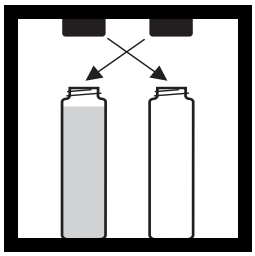
2. Remove the cap from the Bactrol inoculum bottle. Pick out one Bactrol Inoculum Disk with the sterilized forceps.



3. Remove the cap from a Lauryl Tryptose Broth Tube and drop in the Inoculum Disk. Shake to dissolve the disk.



4. Incubate the Lauryl Tryptose Broth Tube until the medium is visibly turbid. Turbidity will develop much faster if incubation is done at 35 °C instead of room temperature. At 35 °C, 10 hours is usually sufficient.



5. Inoculate a new Lauryl Tryptose Broth Tube by first inverting the tube in *step 4*, and then switching the caps of the two tubes. Then invert the new tube. After incubation, use this new vial in subsequent tests.

Note: *In this way, several medium vials may be inoculated from one Bactrol Disk.*

Note: *If testing on consecutive days, keep inoculum several days in the incubator or at room temperature.*

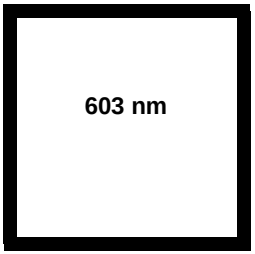
* U.S. Patent 5,413,916

** Liu, D., *Bull Environ. Contam. Toxicol.* 26, 145-149 (1981).

Colorimetric Reaction

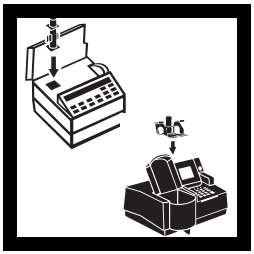
1. Set the instrument up to measure absorbance.

Note: *For the DR/2010, use the constant-on mode and press* **0 ENTER.** *For the DR/4000, press the soft key under* ***SINGLE*** **λ.** *For the DR/800s, press* **PRGM***, then enter* **61***.*



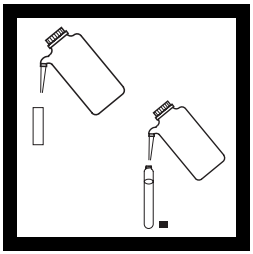
2. Set the wavelength to 603 nm.

Note: *DR/800s will go to 610 nm automatically.*



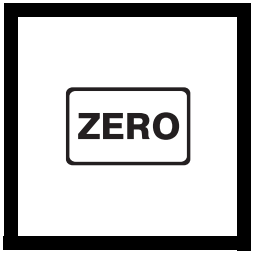
3. Insert the appropriate sample cell adapter into he instrument.

Note: *Use a Test 'N Tube adapter for the DR/2010 and DR/800s. Use a 1-cm square cell adapter for the DR/4000.*



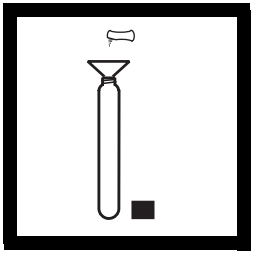
4. Fill a sample cell/tube with deionized water. Label it as "blank". Place the blank into the adapter.

Note: *Use a square 1-cm cells for DR/4000s and a Test 'N Tube vial for the DR/2010 and DR/800s.*

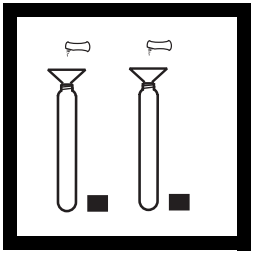


5. Press: **ZERO.** The display will show:

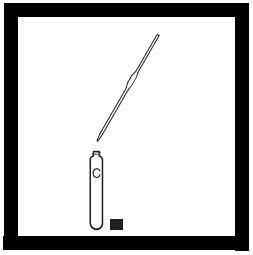
0.000Abs



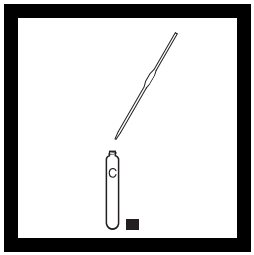
6. Label one sample cell/tube as "control". Open one ToxTrak Reagent Powder Pillow and add the contents to the empty cell/tube.



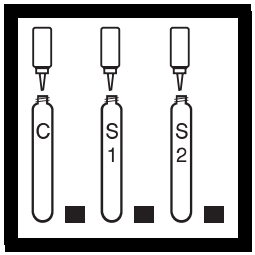
7. For each sample or dilution, repeat *step 6*. Label each cell/tube clearly.



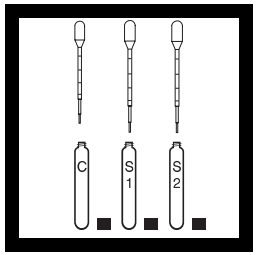
8. Add 5.0 mL of deionized water to the control cell/tube. Cap. Shake all tubes vigorously for 30 seconds.



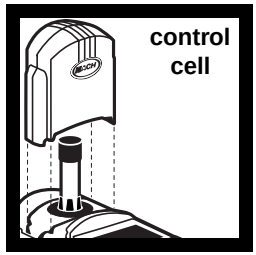
9. Add 5.0 mL of sample (or dilutions) to the sample cell/ tubes.



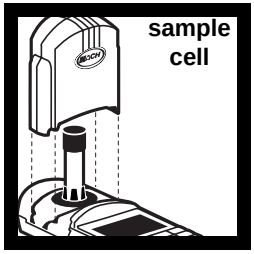
10. Add 3 drops of Accelerator Solution to each cell/tube. Cap and invert to mix.



11. Add 0.5 mL of inoculum (previously prepared) to each cell/tube. Cap and invert to mix.



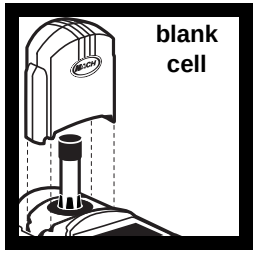
12. Place the control cell/tube in the adapter. Cover the adapter. Record the absorbance.



13. Repeat *step 12* for all samples and dilutions. Be sure to record each absorbance.

14. Allow the solutions to react until the absorbance of the **control tube** decreases 0.60 ±0.10. This should take about 45–75 minutes.

Note: *Do not leave the tubes in the sample compartment during the reaction period.*

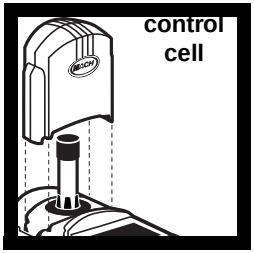


15. Place the blank prepared in *step 4* in the adapter. Cover the adapter.

16. Press: **ZERO**

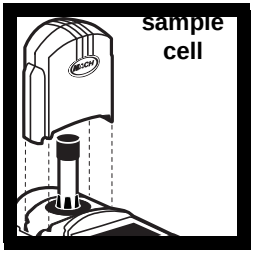
The display will show:

0.000

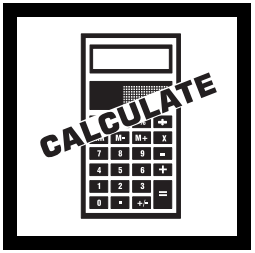


17. Place the control cell/tube into the adapter. Cover the adapter. Record the absorbance value.

Note: *Invert each tube to re-mix before reading it.*



18. Place each sample cell/tube into the adapter. Cover the adapter. Record the absorbance value of each sample.



19. Calculate the % Inhibition as follows:

$$\%I = \left[1 - \left(\frac{\Delta Abs\ sample}{\Delta Abs\ control}\right)\right] \times 100$$

See the example below.

Note: *Some toxins increase respiration and will give a negative % inhibition on all respiration-based toxicity tests. After repeated testing, samples which always give a % inhibition in step 19 that is more negative than 10% should be considered toxic.*

Example:

The control tube (C) has an initial absorbance of 1.6 and decreases to 1.0 Abs. The sample tube has an initial absorbance of 1.7 and decreases to 1.3 Abs.

Abs. Sample $= 1.7 - 1.3 = 0.4$
Abs. Control $= 1.6 - 1.0 = 0.6$

$$\%I = \left(1 - \left(\frac{0.4}{0.6}\right)\right) \times 100$$

$$\%I = 33.3$$

Disposal of Test Cultures

Dispose of active bacterial cultures using one of these methods:

1. Autoclave used test containers at 121 °C for 15 minutes at 15 pounds of pressure. Once the containers are sterile, pour the contents down the drain with running water. The reaction tubes may be washed and re-used.

2. Sterilize test containers by using a 1:10 dilution of commercial laundry bleach. Pour the test container contents and test containers into the bleach solution. Allow 10–15 minutes of contact time with the bleach solution. Pour the liquid down the drain and wash the reaction tubes for re-use.

Summary of Method

This method is based on the reduction of resazurin, a redox-active dye, by bacterial respiration. When it is reduced, resazurin changes color from blue to pink. Toxic substances can inhibit the rate of resazurin reduction. A chemical accelerant has been added to shorten the reaction time.

REQUIRED REAGENTS

Description	Cat. No.
ToxTrak™ Reagent Set (25 tests)	25972-00

Includes: (1) 25607-66, (1) 25608-36, (1) 22336-15, (2) 21247-20, (2) 20962-08

Description	Quantity Required Per Test	Unit	Cat. No.
ToxTrak™ Reagent Powder Pillows	1 pillow	50/pkg	25607-66
ToxTrak™ Accelerator Solution	2 drops	15 mL SCDB	25608-36
Tryptic Soy Broth Tubes	1	15/pkg	22336-15

REQUIRED APPARATUS

Adapter, Test 'N Tube™, DR/2010	1	each	44798-00
Adapter, 1-cm, square, DR/4000	1	each	48584-00
Adapter, Test 'N Tube™, DR/800	1	each	48464-00
Clippers, to open powder pillows	1	each	936-00
Tubes, Test 'N Tube™	varies	10/pkg	20962-08
Dropper Pipet, 1 mL	varies	20/pkg	21247-20
Forceps, flat square tip	1	each	14537-00
Pipet, Volumetric, 5.0 mL, Class A	varies	each	14515-37
Pipet Filler, Safety Bulb	1	each	14651-00
Sample Cell, 1 cm, square,	1	10/pkg	26275-10

OPTIONAL REAGENTS

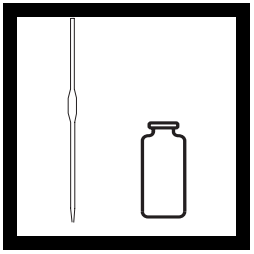
Culture Set (Bactrol Discs & Lauryl Tryptose Broth Tubes)	25 cultures		25978-00
Bactrol Discs, *E. coli*		25/bottle	25809-25
Isopropanol	500 mL		14459-49
Lauryl Tryptose Broth Tubes		15/pkg	21623-15

OPTIONAL APPARATUS

Burner, Alcohol, 60 mL	each	20877-42
Burner, Bunsen	each	21627-00
Germicidal Cloth	50/pkg	24632-00
Incubator, Dri Bath, 25 well, 115-230 V, North American plug	each	45900-00
Incubator, Dri Bath, 25 well, 115-130 V, European plug	each	45900-02
Test Tube Rack	each	24979-00

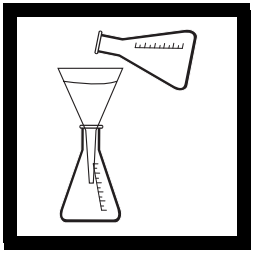
VOLATILE ACIDS

Esterification Method*
Range: 0–2800 mg/L as HOAc



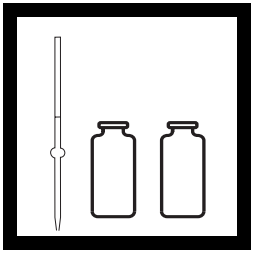
1. Pipet 0.5 mL of deionized water into a dry 25-mL sample cell (the blank).

Note: *The TenSette Pipet may be used for pipetting in this procedure.*



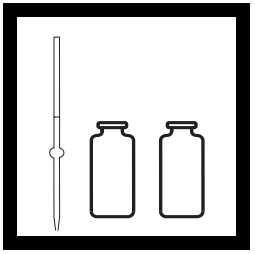
2. Filter or centrifuge 25 mL of the sample.

Note: *Centrifugation is faster than filtration.*

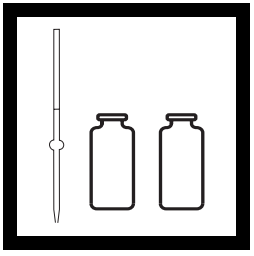


3. Pipet 0.5 mL of the filtrate or supernatant into another dry 25-mL sample cell (the prepared sample).

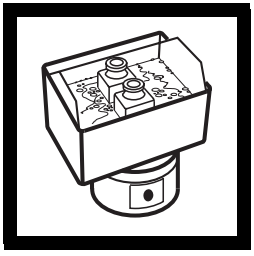
Note: *Use a Class A or TenSette Pipet.*



4. Pipet 1.5 mL of ethylene glycol into each sample cell. Swirl to mix.



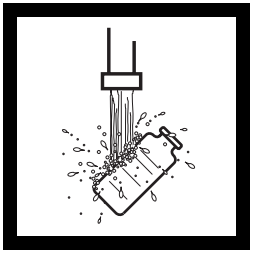
5. Pipet 0.2 mL of 19.2 N Sulfuric Acid Standard Solution into each cell. Swirl to mix.



6. Place both cells into a boiling water bath.

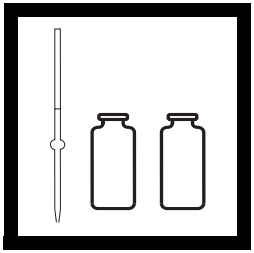
Note: *Samples may be boiled in a 600-mL beaker.*

7. Begin a 3-minute reaction period.

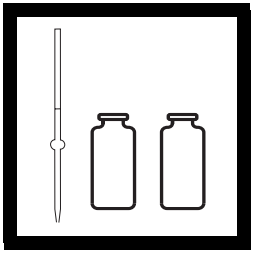


8. When the timer beeps, cool solutions to 25 °C (until cell feels cold) with running tap water.

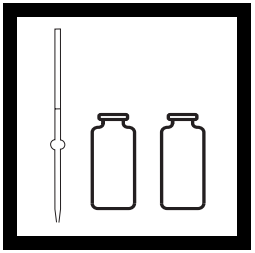
* Adapted from *The Analyst*, 87 949 (1962).



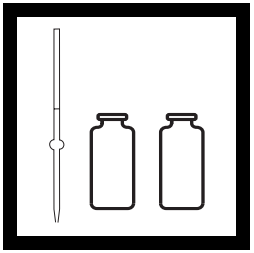
9. Pipet 0.5 mL of Hydroxylamine Hydrochloride Solution into each cell. Swirl to mix.



10. Pipet 2.0 mL of 4.5 N Sodium Hydroxide Standard Solution into each cell. Swirl to mix.



11. Add 10 mL of Ferric Chloride Sulfuric Acid Solution to each cell. Swirl to mix.



12. Add 10 mL of deionized water to each cell. Swirl to mix.

13. Begin a three-minute reaction period.

14. When the timer beeps, zero the instrument with the blank, using the settings below.

DR/800s
Program No. 96

DR/2010
Program No. 770
495 nm

DR/4000
Program No. 3800
495 nm

15. Place the prepared sample into the cell holder. Close the light shield. Read the mg/L volatile acids (as acetic acid).

Sampling and Storage

Collect samples in plastic or glass bottles. Analyze samples as soon as possible after collection. Samples can be stored up to 24 hours by cooling to 4 °C (39 °F) or below. Warm to room temperature before running the test.

Accuracy Check

Standard Additions Method

a. Snap the neck off a Volatile Acids Voluette Ampule Standard Solution, 62,500 mg/L as acetic acid.

b. Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard, respectively, to three 25-mL graduated mixing cylinders, each containing 25 mL of filtered sample. Stopper. Shake well to mix.

c. Remove a 0.5 mL aliquot of sample from each cylinder; add to three dry sample cells. Analyze all three samples along with the original test sample beginning with *step 6* of the procedure. The volatile acid concentration should increase 250 mg/L volatile acids as acetic acid for each 0.1 mL of standard added.

d. If these increases do not occur, an interference is likely.

Standard Solution Method

Prepare a 500 mg/L volatile acid standard by using the TenSette Pipet to add 0.8 mL of a Volatile Acids Voluette Ampule Standard Solution (62,500 mg/L as acetic acid) to a 100-mL volumetric flask. Dilute to volume with deionized water.

Summary of Method

The volatile acids test is designed specifically for the determination of volatile acids in digested sludges. The method is based on esterification of the carboxylic acids present and determination of the esters by the ferric hydroxamate reaction. All volatile organic acids present are reported as their equivalent mg/L acetic acid.

REQUIRED REAGENTS

	Cat. No.
Volatile Acids Reagent Set (90 tests) Includes: (1) 2039-53, (2) 2042-53, (1) 818-42, (1) 2040-53, (1) 2038-32	22447-00

Description	Quantity Required Per Test	Units	Cat. No.
Ethylene, Glycol	3 mL	1000 mL	2039-53
Ferric Chloride-Sulfuric Acid Solution	20 mL	1000 mL	2042-53
Hydroxylamine Hydrochloride Solution, 100 g/L	1 mL	100 mL	818-42
Sodium Hydroxide Standard Solution, 4.5 N	4 mL	1000 mL	2040-53
Sulfuric Acid Standard Solution, 19.2 N	0.4 mL	100 mL MDB	2038-32
Water, deionized	20.5 mL	4 L	272-56

REQUIRED APPARATUS

Cots, finger	2	2/pkg	14647-02
Cylinder, graduated, 10 mL	1	each	508-38
Filter Paper, folded, 12.5 cm	1	100/pkg	1894-57
Flask, Erlenmeyer, 50 mL	1	each	505-41
Funnel, poly, 65 mm	1	each	1083-67
Hot Plate, circular, 10 cm diam., 120 V	1	each	12067-01
Hot Plate, circular, 10 cm diam., 240 V	1	each	12067-02
Pipet Filler, safety bulb	1	each	14651-00
Pipet, serological, 2 mL	2	each	532-36
Pipet, volumetric, Class A, 0.5 mL	3	each	14515-34
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	19700-10
Pipet Tips, for TenSette® Pipet 19700-10	varies	50/pkg	21997-96
Water Bath and Rack	1	each	1955-55

OPTIONAL REAGENTS

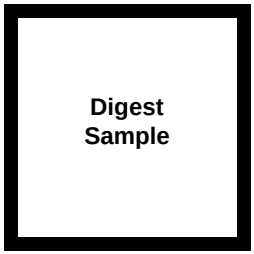
Volatile Acids Standard Solution, Voluette™ ampule, 62,500 mg/L as acetic acid, 10 mL		16/pkg	14270-10

OPTIONAL APPARATUS

Ampule Breaker Kit		each	21968-00
Beaker, 600 mL		each	500-52
Cylinder, graduated, mixing, 25 mL		each	1896-40
Cylinder, graduated, plastic, 250 mL		each	1081-46
Flask, volumetric, Class A, 100 mL		each	14574-42
Pipet, TenSette®, 0.1 to 1.0 mL		each	19700-01
Pipet Tips, for 19700-01 TenSette® Pipet		50/pkg	21856-96

ZINC

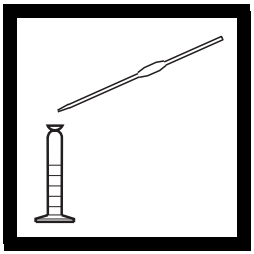
Zincon Method* (USEPA Accepted for wastewater analysis)**
Range: Liquids- 0.3–20000 mg/L; Solids- 20–200000 mg/L



1. Select sample amount from tables following these steps. Digest the sample according to the procedure in *Section 3*.

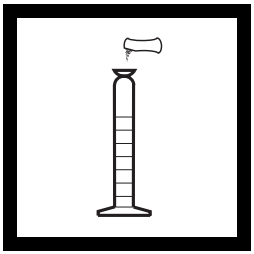
Note: *If sample cannot be analyzed immediately after collection, see* Sampling and Storage *following these steps.*

Note: *The Digesdahl is not a USEPA approved digestion. Samples digested using the Digesdahl may not be used for reporting purposes.*



2. Use the analysis volume in the same table. Pipet the sample analysis volume into a 25-mL mixing cylinder. If the aliquot is more than 0.2 mL, adjust the pH according to the instruction following the digestion method. Dilute to the **20-mL** mark with water.

Note: *Use only glassware and glass stoppers. Wash glassware with 1:1 hydrochloric acid and deionized water before use to avoid errors.*

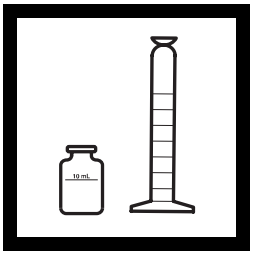


3. Add the contents of one ZincoVer 5 Reagent Powder Pillow to the cylinder. Stopper.

Invert several times to completely dissolve the powder.

Note: *Powder must be completely dissolved.*

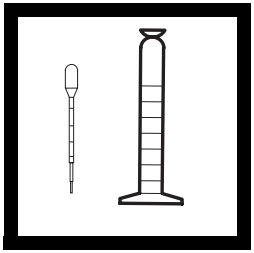
Note: *The sample should be orange. If it is brown or blue, dilute the sample and repeat the test.*



4. Measure 10 mL of the solution into a sample cell (the blank).

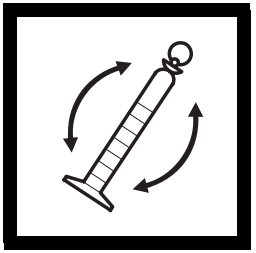
Caution: **ZincoVer 5 contains cyanide and is very poisonous if taken internally or inhaled. Do not add to an acidic sample. Store away from water and acids.**

* Adapted from *Standard Methods for the Examination of Water and Wastewater*
** Federal Register, 45 (105) 36166 (May 29, 1980). Approved only if preceded by an EPA-approved nitric acid digestion. The Digsdahl procedure is not USEPA approved and cannot be used for reporting purposes.



5. Add 0.5 mL of cyclohexanone to the remaining solution in the mixing cylinder.

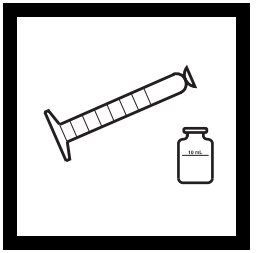
Note: *Use a plastic dropper as rubber bulbs may contaminate the cyclohexanone.*



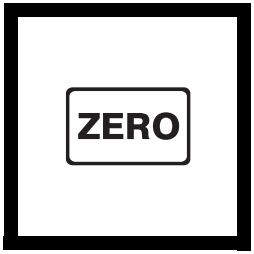
6. Stopper the cylinder. Shake vigorously for 30 seconds (the prepared sample).

Note: *The sample will be red-orange, brown or blue, depending on the zinc concentration.*

7. Begin a three-minute reaction period.



8. During the reaction period pour the solution from the cylinder into a 10-mL sample cell.

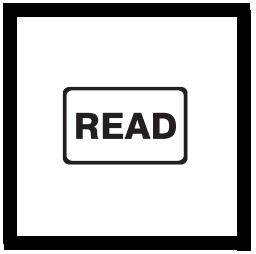


9. After the timer beeps, zero the instrument with the blank, using the settings below.

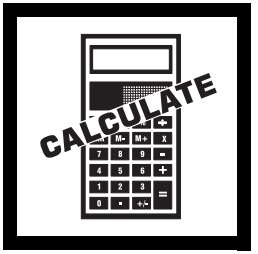
DR/800s
Program No. 97

DR/2010
Program No. 780
620 nm

DR/4000
Program No. 3850
620 nm



10. Place the sample in the cell holder. Read the mg/L zinc.



11. Use the equation below the Zinc Sample and Analysis Volume Tables to calculate the true zinc concentration.

Note: *For solid samples, express results as mg/kg, not mg/L.*

Sample and Analysis Volume Tables

Choose sample and analysis volumes based on expected concentration in the sample. The values in these tables reflect the narrowest concentration range for the DR/4000, DR/2010 and DR/800s. Differences between these tables and those in the *Digesdahl Manual* are due to calculations based on a different set of instruments.

Liquids

Expected Zn Conc. (mg/L)	Sample Amount (mL)	Analysis Volume (mL)	Dilute To
0.3–12.5	40.0	8.00	20 mL
1–50	20.0	4.00	20 mL
4–200	10.0	2.00	20 mL
40–2000	5.00	0.40	20 mL
400–20000	1.00	0.20	20 mL

Solids

Expected Zn Conc. (mg/kg)	Sample Amount (g)	Analysis Volume (mL)	Dilute To
20–1000	0.500	8.00	20 mL
50–2500	0.400	4.00	20 mL
170–6600	0.300	2.00	20 mL
1000–50000	0.200	0.40	20 mL
4000–200000	0.100	0.20	20 mL

Calculation For Final Concentration:

$$\frac{A \times 2000}{B \times C} = \text{ mg/L or mg/kg Total Zn}$$

A = mg/L reading from instrument

B = g or mL sample amount from table

C = mL analysis volume from table

Sampling and Storage

Collect samples in acid-washed plastic bottles. For storage, adjust the pH to 2 or less with nitric acid (about 2 mL per liter). The preserved samples can be stored up to six months at room temperature.

Adjust the pH to 4–5 with 5.0 N sodium hydroxide before analysis. Do not exceed pH 5, as zinc may be lost as a precipitate. Correct the test result for volume additions.

If only dissolved zinc is to be determined, filter the sample before adding the acid.

Accuracy Check

Standard Addition Method

a. Perform the zinc method and note the analysis volume and the mg/L Zn.

b. Pipet the same analysis volume into three 25-mL graduated mixing cylinders.

c. Snap the neck off a Zinc Voluette Ampule Standard, 25 mg/L.

d. Use the TenSette Pipet to add 0.2, 0.4, and 0.6 mL of standard to the three samples. Dilute to the 20-mL mark. Mix each thoroughly.

e. Analyze each sample as described above. The zinc concentration should increase 0.25 mg/L for each 0.2 mL of standard added.

f. If these increases do not occur, an interference is likely.

Standard Solution Method

Prepare a 0.5 mg/L zinc standard solution by diluting 0.5 mL of Zinc Standard Solution, 100 mg/L as Zn, to 100 mL with deionized water. Prepare this solution daily. Perform the zinc method as described in *steps 3–12*. The result should be 0.5 mg/L.

Interferences

The following may interfere when present in concentrations exceeding those listed below.

Substance	Concentration
Aluminum	6 mg/L
Cadmium	0.5 mg/L
Copper	5 mg/L*
Iron (ferric)	7 mg/L*
Manganese	5 mg/L
Nickel	5 mg/L

* Monitor copper and iron by diluting the final sample 1:2 and using Hach Water Quality Copper Test Strips (Cat. No. 27451-25) and Hach Water Quality Iron Test Strips (Cat. No.27453-25).

Pollution Prevention and Waste Management

ZincoVer 5 reagent contains potassium cyanide, which is regulated as hazardous wastes by the Federal RCRA. Cyanide should be collected for disposal as reactive (D003) waste. Be sure that cyanide solutions are stored in a caustic solution with pH >11 to prevent the release of hydrogen cyanide gas.

In the event of a spill or release, clean up the area by using the following procedure:

a. Use a fume hood, supplied-air, or self-contained breathing apparatus.

b. While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and calcium hypochlorite or sodium hypochlorite (household bleach).

c. Maintain a strong excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.

d. Neutralize and flush the solution down the drain with a large excess of water.

Summary of Method

Zinc and other metals in the sample are complexed with cyanide. Adding of cyclohexanone causes a selective release of zinc. Zinc then reacts with the 2-carboxy-2′-hydroxy-5′-sulfoforamazyl benzene (zincon) indicator. The zinc concentration is proportional to the resulting blue color.

REQUIRED REAGENTS

	Cat. No.
Zinc Reagent Set, 10-mL sample size (100 tests) Includes: (1) 14033-32, (1) 21066-69	24293-00

Description	Quantity Required Per Test	Units	Cat. No.
Cyclohexanone	1 mL	100 mL MDB	14033-32
ZincoVer® 5 Reagent Powder Pillows	1 pillow.	100/pkg	21066-69
Water, deionized		4 L	272-56

REQUIRED APPARATUS

Cylinder, graduated, mixing, 25 mL	1	each	20886-40
Pipet, volumetric, Class A, 8.00 mL	1	each	14515-08
Pipet, volumetric, Class A, 4.00 mL	1	each	14515-04
Pipet, volumetric, Class A, 2.0 mL	1	each	14515-36
Pipet, volumetric, Class A, 0.5 mL	1	each	14515-34

OPTIONAL REAGENTS

Bleach, household	1 gal	buy locally
Hydrochloric Acid Standard Solution, 6 N	500 mL	884-49
Nitric Acid, ACS	500 mL	152-49
Nitric Acid 1:1	500 mL	2540-49
Sodium Hydroxide Standard Solution, 5.0 N	50 mL SCDB	2450-26
Sodium hydroxide, 50% w/w	500 mL	2180-49
Water Quality Test Strips, total copper	25/pkg	27451-25
Water Quality Test Strips, total iron	25/pkg	27453-25
Zinc Standard Solution, 100 mg/L Zn	100 mL	2378-42
Zinc Standard Solution, Voluette™ ampule, 25 mg/L as Zn, 10 mL	16/pkg	14246-10

APPENDIX A TROUBLESHOOTING

1. If the results from any Hach chemistry are in doubt, follow the instructions below:

a. Run a proof-of-accuracy check with a standard solution. Take a known standard solution through all the same steps as the original sample in question, including sampling, storage, digestion, and the colorimetric procedure. If the results on the standard solution are correct, proceed to *step 3*. If the results differ significantly from the standard concentration, proceed to *step 2*.

2. If the results of the standard solution accuracy check do not match expected results, the reagents may need replacing or the analysis technique may be incorrect. Proceed to *step a*.

a. Determine the age of the reagents used. Most Hach reagents are stable for at least one year, but many factors affect shelf life (heat, microbial contamination, humidity). If one reagent is suspect, replace that chemical and run the standard accuracy check again.

b. Run a deionized water blank through all portions of the procedures, including sampling, storage, digestion, and the colorimetric procedure. Some chemicals add a small amount of color to a test, but this is not considered unusual. Look for color development in excess of 10% of the range of the test. This can indicate a problem with one of the reagents or the dilution water.

c. If the reagents seem to be working correctly, troubleshoot the processing phases the sample goes through. First, omit the storage/preservation phase and run the digestions and colorimetric method on a standard solution. If this test gives the correct result, examine the storage procedure. Make sure to follow the storage instructions given in each procedure. Be sure to acidify the sample with the correct acid to the correct pH and to neutralize the sample to the proper pH before testing.

If the standard check still results in an incorrect value, omit the digestion phase and run the colorimetric test on a standard. If this value is correct, examine the digestion

procedure. Make sure correct amounts of acid and peroxide are used and that the pH adjustment following the digestion is followed. Refer to the procedure for the specific parameter to confirm the correct reagents are used.

3. If the standard accuracy check from *step a* gives a correct value, but the sample results are questionable, an interfering substance may be present in the sample. Troubleshoot the interference as follows:

a. Run a standard spike on the sample. This provides more complete information about possible problems than a single standard solution check because the analysis includes any interferences that may be present in the sample.

b. Split a fresh sample into two equal aliquots. Add a known amount of standard solution to one of them. Analyze both the samples using the same reagents, instruments, and techniques. The concentration increase should equal the amount of analyte added. See *Standard Additions* under the *Accuracy Check* section in each procedure.

c. Calculate the percent recovery using these steps:

1. Measure the unknown sample concentration.

2. Calculate the theoretical concentration of the spiked sample as follows:

$$\text{Theoretical concentration} = \frac{(C_u \times V_u) + (C_s \times V_s)}{V_u + V_s}$$

Where:

C_u = measured concentration of the unknown sample
V_u = volume of the unknown sample
C_s = concentration of the standard
V_s = volume of the standard

3. Measure the spiked sample concentration.

4. Divide the spiked sample concentration by the theoretical concentration.

5. Multiply the results from *step 4* by 100. the result is the percent recovery.

Example

Sample result = 4.5 mg/L manganese
A separate 97-mL portion of sample was spiked with 3 mL of a 100-mg/L manganese standard. Using the same procedure, the spiked sample result = 7.1 mg/L.

The theoretical concentration of the spike sample is:

$$\frac{(4.5\ \text{mg/L} \times 97\ \text{mL}) + (3\ \text{mL} \times 100\ \text{mg/L})}{97\ \text{mL} + 3\ \text{mL}} = 7.4\ \text{mg/L}$$

The percent spiked recovery is:

$$\frac{7.1\ \text{mg/L}}{7.4\ \text{mg/L}} \times 100 = 96\%$$

d. Perform a series of dilutions on the sample. Because it may not be possible to determine what the interfering substance is, diluting the sample past the point of interference may be the most economical means of getting a correct result.

e. Make sure the analyte in the sample is within the test range. High analyte levels can cause erroneous results due to over developed or under developed color, excess turbidity, or sample bleaching. Run a series of dilutions to check for this possibility.

f. Sometimes it is not possible to dilute the sample enough to stop the interference. In this case, try to use a different method to measure the parameter, such as a different chemistry or an ion-selective electrode.

Frequently Asked Questions

Off-scale Reading

If the reading on your instrument is off-scale, check the concentration range in the tables following the procedure steps. Use a smaller analysis volume and repeat the procedure (the digestion does not need to be repeated unless there is not enough digestate to repeat the colorimetric phase). Record the new analysis volume and use it in the calculation.

Reagent Blanks

First, determine any contribution the reagent blank makes to the sample result by standardizing the instrument on distilled water. If the blank has an insignificant value and you are using reagents with the same lot number, a reagent blank does not need to be run with every analysis. If the blank contributes to the sample reading, analyze a blank daily or subtract the reading from the sample reading. If a daily reagent blank is not run, standardize the instrument with deionized water.

Sample Amounts and Analysis Volumes

The sample amount and analysis volume in the tables included with each procedure are suggested guidelines. Any aqueous solution or suspension up to 40 mL may be digested. Solid or organic liquid samples require less that 0.5 g of anhydrous material. Generally, 0.25 g of sample is used.

The amount of sample to be digested is critical. The volume of the digestate used for analysis is also very important. The tables following procedure steps can help determine these volumes. Use the following procedure to optimize these amounts.

1. Determine the approximate concentration of the sample.

2. Determine the range of the colorimetric test being used and the midpoint of the test range. The midpoint is optimum, but can be lowered for very low analyte concentrations. Determine the midpoint by subtracting the lower limit of the range from the higher limit of the range and divide by 2.

3. Use the following equations to refine sample and analysis volumes.

$$A = \frac{B \times C \times D}{E \times F}$$

Where:

A = Approximate sample concentration

B = midpoint of colorimetric test range

C = Final volume of the digestate

D = final analysis volume

E = sample amount to digest

F = analysis volume of digestate

Using algebra, these two equations are obtained:

equation (1)

$$E = \frac{B \times C \times D}{A \times F}$$

equation (2)

$$F = \frac{B \times C \times D}{A \times E}$$

Both equations contain two unknown values (E and F), and some trial and error may be required to achieve the optimum values.

Example 1
Analyze for copper using the CuVer method and he DR/2010 spectrophotometer with an initial sample containing 150 mg/L copper. Determine the amount of sample required for digestion and the analysis volume.

In this case the test range is 0 to 5.00 mg/L, and the midpoint is 2.5 mg/L.

When using the Digesdahl, the final volume of digestate is 100 mL and the analysis volume is 25 mL.

So:

A = 150

B = 2.5

C = 100

D = 25

E and F are unknown

Substitute these values into equation 1:

$$E = \frac{2.5 \times 100 \times 25}{150 \times F}$$

$$E = \frac{41.7}{F}$$

Because CuVer Copper Reagent is pH sensitive, a small analysis volume (0.5 mL) is best so a pH adjustment is not necessary. With this in mind, a 0.5-mL analysis volume would give:

$$E = \frac{41.7}{0.5} = 83.4 \text{ mL sample digestion volume}$$

Since the maximum digestion amount is 40 mL, a 0.5-mL analysis volume is not acceptable. Try a 5.0-mL analysis volume:

$$E = \frac{41.7}{5.0} = 8.0 \text{ mL sample digestion volume}$$

(round to the nearest whole number for easy measurement)

An 8.0-mL sample would be digest and a 5.0-mL volume would be taken for colorimetric analysis. A pH adjustment would be necessary before analysis.

Example 2
Use equation 2 when a minimum sample size is desired or when a sample has already been digested for one parameter (such as copper) and another parameter (such as zinc) also needs to be measured.

The digestate from the above example contains 3 mg/L zinc and the Zincon methods is being used. Determine the analysis volume as follows.

The midpoint of the Zincon test range is 1.25 mg/L.

So:

A = 3

B = 1.25

C = 100

D = 50

E = 8 (as determined above)

F = unknown

Substituting these values into equation 2 gives:

$$F = \frac{1.25 \times 100 \times 50}{3 \times 8} = 260 \text{ mL}$$

This is an extreme example, but it illustrates the need to compare the values of D and F to assure that the volume of sample to be digested(F) does not exceed the final analysis volume (D). The analysis cannot be done if F exceeds D. If this occurs, use a test with a more appropriate range or digest a large sample volume.

Be sure that the volume of digestate taken for analysis (F) is greater than 0.1 mL because accurately pipetting less than 0.1 mL is difficult.

As a comparison, assume the zinc concentration is 75 mg/L (A = 75 instead of 3). Substituting into *equation 2* gives:

$$F = \frac{1.25 \times 100 \times 50}{75 \times 8} = 10.5 \text{ mL}$$

Here, the volume of sample to be digested is less than the final analysis volume and the analysis my be done as stated in the procedure.

Calculation Factors of 75, 1000, 2000, 2500, and 5000

In all cases, the factor is a correction for the sample dilution. For example, the factor is 2500 for the iron test. The Digesdahl digestion total volume is 100 mL and the analysis total volume is 25 mL.

So:

$$100 \times 25 = 2500$$

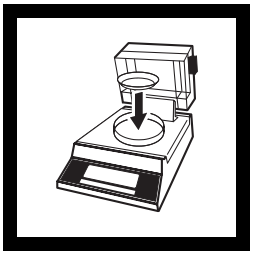
The mL units are not included with the factor because they cancel out in the formula.

The factor 75 is an exception and is due to the fact that the instrument programs were setup using a 25 mL digestion volume and a 3 mL sample for analysis.

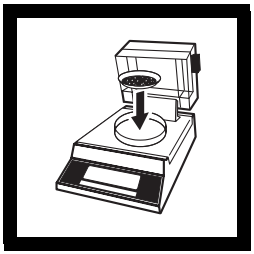
Reporting on a "Dry Basis"

Sometimes it is necessary to report a total concentration on a dry basis when analyzing a slurry. Analyze the sample for moisture content following the procedure on the next page to express results on a dry basis.

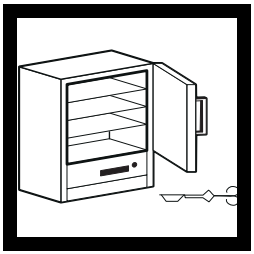
Analysis For "Dry Basis"



1. Weigh an aluminum dish and record the weight.

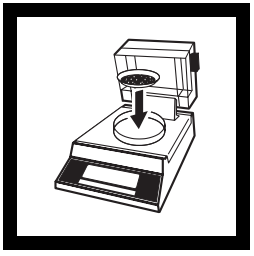


2. Weigh out about 2 g of sample in the dish. Record the exact weight added.



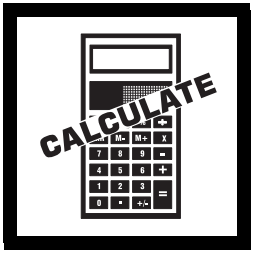
3. Place the dish in an over (103-105 °C) for two hours.

4. Cool to room temperature by placing the dish in a desiccator.



5. Weigh the aluminum dish with the dried sample in it.

Note: *The oven-dried sample generally is unsuitable for additional testing and should be discarded.*



6. Use the following formula to calculate the sample "dry basis".

Test results as dry basis =

$$\frac{A \times B}{C}$$

Where:
A = total metal concentration.
B = net weight of sample taken for oven drying.
C = net weight of oven-dried sample.

Containers, Preservation Techniques and Holding Times

This table is adapted from Table II published in the *Federal Register*, July 1, 1997, 40 CFR, Part 136.3, pages 26–28. Organic tests are not included.

Parameter No./Name	Container[1]	Preservation[2,3]	Maximum Holding Time[4]
Table 1A Bacterial Tests			
1–4. Coliform, fecal and total	P,G	Cool, 4 °C, 0.008%, $Na_2S_2O_3$[5]	6 hours
5. Fecal streptococci	P,G	Cool, 4 °C, 0.008%, $Na_2S_2O_3$[5]	6 hours
Table 1B Inorganic Tests			
1. Acidity	P, G	Cool, 4 °C	14 days
2. Alkalinity	P, G	Cool, 4 °C	14 days
4. Ammonia	P, G	Cool, 4 °C, H_2SO_4 to pH<2	28 days
9. Biochemical oxygen demand (BOD)	P, G	Cool, 4 °C	48 hours
10. Boron	P, PFTE or quartz	HNO_3 to pH<2	6 months
11. Bromide	P, G	None required	28 days
14. Biochemical oxygen demand, carbonaceous	P, G	Cool, 4 °C	48 hours
15. Chemical oxygen demand	P, G	Cool, 4 °C, H_2SO_4 to pH<2	28 days
16. Chloride	P, G	None required	28 days
17. Chlorine, total residual	P, G	none required	Analyze immediately
21. Color	P, G	Cool, 4 °C	48 hours
23-24. Cyanide, total and amenable to chlorination	P, G	Cool, 4 °C, NaOH to pH>12, 0.6 g ascorbic acid[6]	14 days
25. Fluoride	P	None required	28 days
27. Hardness	P, G	HNO_3 to pH<2, H_2SO_4 to pH<2	6 months
28. Hydrogen ion (pH)	P, G	None required	Analyze immediately
31, 43. Kjeldahl and organic nitrogen	P, G	Cool 4 °C, H_2SO_4 to pH<2	28 days

Parameter No./Name	Container[1]	Preservation[2,3]	Maximum Holding Time[4]
Metals[7]			
18. Chromium VI	P, G	Cool, 4 °C	24 hours
35. Mercury	P, G	HNO_3 to pH<2	6 months
Metals, except boron, chromium VI and mercury. 3, 5–8, 12, 13, 19, 20, 22, 26, 29, 30, 32-34, 36, 37, 45, 47, 51, 52, 58-60, 62, 63, 70–72, 74, 75[8].	P, G	HNO_3 to pH<2	6 months
38. Nitrate	P, G	Cool, 4 °C	48 hours
39. Nitrate-nitrite	P, G	Cool 4°C, H_2SO_4 to pH<2	28 days
40. Nitrite	P, G	Cool, 4 °C	48 hours
41. Oil and grease	G	Cool, 4 °C, HCl or H_2SO_4 to pH<2	28 days
42. Organic Carbon	P, G	Cool, 4 °C, HCl or H_2SO4 or H_3PO_4 to pH<2	28 days
44. Orthophosphate	P, G	Filter immediately; Cool, 4°C	48 hours
46a. Oxygen, dissolved probe	G Bottle and top	None required	Analyze immedi-ately
46b. Oxygen, dissolved, Winkler	G Bottle and top	Fix on site and store in dark	8 hours
48. Phenols	G only	Cool 4 °C, H_2SO_4 to pH<2	28 days
49. Phosphorus, elemental	G	Cool, 4 °C	48 hours
50. Phosphorus, total	P, G	Cool, 4°C, H_2SO_4 to pH<2	28 days
53. Residue, total	P, G	Cool, 4 °C	7 days
54. Residue, filterable	P, G	Cool, 4 °C	7 days
55. Residue, Nonfilterable (TSS)	P, G	Cool, 4 °C	7 days
56. Residue, Settleable	P, G	Cool, 4 °C	48 hours
57. Residue, volatile	P, G	Cool, 4 °C	7 days
61. Silica	P, PFTE or quartz	Cool, 4 °C	28 days
64. Specific conductance	P, G	Cool, 4 °C	28 days
65. Sulfate	P, G	Cool, 4 °C	28 days
66. Sulfide	P, G	Cool 4 °C, add zinc acetate plus sodium hydroxide to pH>9	7 days

Parameter No./Name	Container[1]	Preservation[2,3]	Maximum Holding Time[4]
67. Sulfite	P, G	none required	Analyze immediately
68. Surfactants	P, G	Cool, 4 °C	48 hours
69. Temperature	P, G	None required	Analyze immediately
73. Turbidity	P, G	Cool, 4 °C	48 hours

1 . Polyethylene (P) or glass (G)

2 . Sample preservation should be performed immediately upon sample collection. For composite chemical samples each aliquot should be preserved at the time of collection. When use of an automated sampler makes it impossible to preserve each aliquot, then chemical samples may be preserved by maintaining at 4 °C until compositing and sample splitting is completed.

3 . When any sample is to be shipped by common carrier or sent through United States Mails, it must comply with the Department of Transportation Hazardous Material Regulations (49 CFR Part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO_3) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H_2SO_4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH < 12.30).

4 . Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still be considered valid. Samples may be held for longer periods only if the permitee, or monitoring laboratory, has data on file to show that the specific types of samples under study are stable for the longer time, and has received a variance from the Regional Administer under §136.3(e). Some samples may not be stable for the maximum time period given in the table. A permitee, or monitoring laboratory, is obligated to hold the sample for a shorter time if knowledge exists to show that this is necessary to maintain sample stability. See §136.3(e) for details. “Analyze immediately” usually means within 15 minutes or less after sample collection.

5 . Should only be used in the presence of residual chlorine

6 . Maximum holding time is 24 hours when sulfide is present. Optionally all samples may be tested with lead acetate paper before pH adjustments in order to determine if sulfide is present. If sulfide is present, it can be removed by the addition of cadmium nitrate powder until a negative spot test is obtained. The sample is filtered and then NaOH is added to pH 12.

7 . For dissolved metals, samples should be filtered immediately on-site before adding preservative.

8 . Numbers refer to parameter number in 40 CFR, Part 136.3, Table 1B.

Related Literature

Hach has several other methods and reference materials related to biosolids and wastewater analysis. Please contact a Hach Customer Service Representative at 800-227-4224 to request the literature below. Refer to the Lit. # when requesting the material.

1. Microbiology Systems For Analysis Catalog (Lit. # 3986) — contains a complete description of Hach microbiological products.

2. Microbiological Laboratory Start-Up Guide (Lit. # 7047) — Contains streamlined information on products for a typical lab start-up.

3. Coliform Multiple Tube Fermentation Technique (Lit. # 8368) — Contains procedures for use with Hach prepared media.

4. Detecting Fecal Coliforms in Sludge (Lit. # 8391) — Procedure for use with Hach A-1 Medium Broth.

Tests Performed by Hach Photometers

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Aluminum, Aluminon Method	yes	0-0.80	10	522	0-1.000	2	522	0-0.800	1000	522	0-0.8	1	520
Aluminum, ECR Method		0-0.220	9	535	0-0.250	102	535	0-0.250	1010	535	N/A	---	---
Ammonia (See Nitrogen Ammonia)													
Arsenic		0-0.200	9--	520	0-0.2	1	520	0-0.200	1050	520	N/A	---	---
Barium		0-100	20	450	N/A	---	---	0-100	1100	450	N/A	---	---
Barium (AccuVac)		0-100	25	450	N/A	---	---	0-100	1110	450	N/A	---	---
Benzotriazole		0-16.0	30	425	0-15.0	3	425	0-16.0	1200	425	0-16.0	3	420
Boron		0-14.0	40	605	0-20.0	5	605	0-14.0	1250	605	0-16.0	4	610
Boron, LR		0-1.5	45	410	0-15.0	N/A	410	0-1.5	1260	410	N/A	---	---
Bromine	yes	0-4.50	50	530	0-4.00	6	530	0-4.50	1300	530	0-4.50	5	525
Bromine (AccuVac)	yes	0-4.50	55	530	0-4.00	80	530	0-4.50	1310	530	0-4.50	52.04.1	525
Cadmium (Dithizone)		0-80 µg/L	60	515	0-180 µg/L	7	515	0-80.0 µg/L	1350	515	N/A	---	---
Calcium (See Hardness, Calcium)													
Chloride		0-20.0	70	455	0-25	73	455	0-25.0	1400	455	N/A	---	---
Chlorine, Ultra Low Range for wastewater	DR/2000 DR/2010	0-500 µg/L 0-500 µg/L	88 86	530 515	0-500 µg/L	N/A	510	0-500 µg/L	1490	515	N/A	---	---
Chlorine, Ultra Low Range for clean water	DR/2000 DR/2010	0-500 µg/L 0-500 µg/L	88 86	530 515	0-500 µg/L	N/A	510	0-500 µg/L	1490	515	N/A	---	---
Chlorine, Free	yes	0-2.0	80	530	0-1.70	8	530	0-2.00	1450	530	0-2.00	9	520
Chlorine, Free (AccuVac)	yes	0-2.00	85	530	0-2.00	81	530	0-2.00	1460	530	0-2.00	11	520
Chlorine, Free, HR		0-5.00 ‘	1	530	0-5.00	N/A	530	0-5.00	1470	530	0-5.00	8	520
Chlorine, Total	yes	0-2.00	80	530	0-1.70	8	530	0-2.00	1450	530	0-2.00	9	520
Chlorine, Total (AccuVac)	yes	0-2.00	85	530	0-2.00	81	530	0-2.00	1460	530	0-2.00	11	525

Tests Performed by Hach Photometers (Continued)

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Chlorine, Total, HR		0-5.00	88	530	0-5.00	N/A	530	0-5.00	1470	530	0-5.00	8	520
Chlorine Dioxide, High Range		0-700	75	445	0-700	11	445	0-1000	1520	445	N/A	---	---
Chlorine Dioxide, Medium Range		N/A	---	---	0-50.0	10	360	0-50	1510	360	0-50.0	7	420
Chlorine Dioxide, Low Range		0-1.00	72	575	0-1.00	9	575	0-1.0	1500	575	N/A	---	---
Chlorine Dioxide, DPD	yes	0-5.00	76	530	0-5.00	manual	530	0-5.00	1530	530	0-5.00	112	530
Chlorine Dioxide, DPD, AccuVac	yes	0-5.00	77	530	0-5.00	manual	530	0-5.00	1535		0-5.00	113	530
Chlorophyll a		N/A	---	---	N/A	---	---	varies	Manual	several	N/A	---	---
Chromium, Hexavalent	yes	0-0.60	90	540	0-1.00	12	540	0-0.70	1560	540	0-0.60	13	560
Chromium, Hexavalent (AccuVac)	yes	0-0.60	95	540	0-1.00	82	540	0-0.70	1570	540	0-0.60	14	560
Chromium, Total		0-0.60	100	540	0-0.70	13	540	0-0.700	1580	540	0-0.600	15	560
Chromium, Trivalent (not in DR/2010)		0-20.0 g/L	105	595	0-20.0 g/L	14	595	0-20. 0 g/L	1550	595	N/A	---	---
Cobalt		0-2.00	110	620	0-2.00	15	620	0-2.00	1600	620	N/A	---	---
COD (see Oxygen Demand, Chemical)													
Color, ADMI		N/A	----	---	N/A	---	---	0-250 units	1660	700	N/A	---	---
Color, APHA Pt-Co		0-500 units	120	455	0-500 units	16	455	0-500 units	1670	455	0-500 units	19	420
Color, NCASI 253		0-500 units	125	465	N/A	---	---	0-500 units	1680	465	N/A	---	---
Color, Tristimulus & Chromicity		N/A	---	---	N/A	---	---	N/A	1666	780	N/A	---	---
Color, Yellowness		N/A	---	---	N/A	---	---	N/A	1668	780	N/A	---	---
Color, Gardner		N/A	---	---	N/A	---	---	N/A	1664	780	N/A	---	---
Copper, Autocatalytic		0-3.00 g/L	9--	810	0-3 g/L	N/A	810	0-3.00 g/L	1690	810	N/A	---	---

Tests Performed by Hach Photometers (Continued)

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Copper, Bicinchoninate Method	yes	0-5.00	135	560	0-5.00	17	560	0-5.000	1700	560	0-5.00	20	550
Copper, Bicinchoninate Method (AccuVac)	yes	0-5.00	140	560	0-5.00	83	560	0-5.000	1710	560	0-5.00	20	550
Copper, Porphyrin		0-210 µg/L	145	425	0-250 µg/L	18	425	0-210.0 µg/L	1720	425	0-210 µg/L	22	420
Cyanide		0-0.200	160	612	0-0.240	19	612	0-0.240	1750	612	0-0.240	21	610
Cyanuric Acid		0-50	170	480	N/A	---	---	N/A	---	---	7-55	24	520
DEHA (Diethyl-hydroxylamine)		0-450 µg/L	180	562	0-600 µg/L	20	562	0-500 µg/L	2738	562	0-500 µg/L	25	560
Detergents (See Surfactants)													
Dissolved Oxygen (See Oxygen, Dissolved)													
Erythorbic Acid (See DEHA)		0-1500 µg/L	180	562	0-2100 µg/L	20	562	0-1500 µg/L	2742	562	See DEHA	---	---
Fluoride	yes	0-2.00	190	580	0-1.50	22	580	0-2.00	1900	580	0-2.00	27	610
Fluoride (AccuVac)	yes	0-2-4	195	580	0-2-4	79	580	0-2.00	1910	580	0-2.00	28	610
Formaldehyde		0-350 µg/L	200	630	0-1300 µg/L 0-500 µg/L	64 65	630 630	0-500 µg/L	1950	630	N/A	---	---
Hardness, Calcium		0-1.6	221	522	0-1.0	63	522	0-4.00	2020	522	0-4.00	30	520
Hardness, Calcium as $CaCO_3$		0-4.0	220	522	0-2.5	63	522	0-4.00	2020	522	N/A	---	---
Hardness, Magnesium		0-1.0	226	522	0-0.48	62	522	0-4.00	2020	522	0-4.00	30	520
Hardness, Magnesium as $CaCO_3$		0-4.0	225	522	0-2.0	62	522	0-4.00	2020	522	N/A	---	---
Hardness, Ultra Low Range		0-1000 µg/L	227	669	0-1000 µg/L	N/A	669	0-1000 µg/L	2000	669	N/A	---	---

Tests Performed by Hach Photometers (Continued)

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Hydrazine		0-500 µg/L	231	455	0-600 µg/L	23	455	0-600 µg/L	2050	455	0-500 µg/L	31	420
Hydrazine (AccuVac)		0-500 µg/L	232	455	N/A	---	---	0-600 µg/L	2060	455	0-500 µg/L	32	420
Hydroquinone		0-1125 µg/L	180	562	0-1500 µg/L	20	562	0-1000 µg/L	2740	562	N/A	---	---
Iodine		0-7.00	240	530	0-6.00	24	530	0-7.00	2100	530	N/A	---	---
Iodine (AccuVac)		0-7.00	242	530	0-6.00	84	530	0-7.00	2110	530	N/A	---	---
Iron, Ferrous		0-3.00	255	510	0-3.000	25	510	0-3.00	2150	510	0-3.00	33	520
Iron, Ferrous (AccuVac)		0-3.00	257	510	0-3.000	93	510	0-3.00	2155	510	0-3.00	33	520
Iron, Total, FerroMo		0-1.80	275	590	0-1.80	77	590	0-1.800	2160	590	0-1.30	38	610
Iron, FerroZine Method		0-1.300	260	562	0-1.400	27	562	0-1.400	2175	562	0-1.300	37	560
Iron, Total, FerroVer Method		0-3.00	265	510	0-3.000	26	510	0-3.00	2165	510	0-3.00	33	520
Iron, Total, FerroVer Method (AccuVac)		0-3.00	267	510	0-3.000	85	510	0-3.00	2170	510	0-3.00	33	520
Iron, Total, TPTZ Method	yes	0-1.80	270	590	0-1.000	77	590	0-1.800	2190	590	0-1.80	39	610
Iron, Total, TPTZ Method (AccuVac)	yes	0-1.80	272	590	0-1.000	86	590	0-1.800	2195	590	0-1.80	39	610
Lead, Dithizone Method		0-160 µg/L	280	515	0-300 µg/L	28	515	0-300 µg/L	2200	515	N/A	---	---
Lead, LeadTrak Fast Column Extraction		0-150 µg/L	283	477	0-150 µg/L	99	477	0-150 µg/L	2210	477	N/A	---	---
Magnesium (See Hardness, Magnesium)													
Manganese, High Range	yes	0-20.0	295	525	0-20.0	29	525	0-20.0	2250	525	0-20.0	41	520
Manganese, Low Range (PAN)	yes	0-0.700	290	560	0-0.800	30	560	0-0.700	2260	560	0-0.700	43	560

Tests Performed by Hach Photometers (Continued)

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Methylethylketoxime (See DEHA)		0-1575 µg/L	180	562	0-2100 µg/L	20	562	0-1000 µg/L	2744	562	See DEHA		
Mercury, Cold Vapor Method		0.1-2.5 µg/L	312	412	0.1-2.5 µg/L	N/A	412	0.1-2.5 µg/L	2270	412	N/A	---	---
Molybdenum, High Range (as Mo)		0-35.0	320	420	0-20.0	31	365	0-50.0	2310	420	0-40.0	44	420
Molybdenum, High Range (AccuVac)		0-35.0	322	420	0-25.0	N/A	365	0-50.0	2320	420	0-40.0	44	420
Molybdenum, Low Range (as Mo)	yes	0-3.00	315	610	0-3.00	94	610	0-3.00	2300	610	0-3.00	47	610
Nickel, Autocatalytic		0-8.00 g/L	330	720	0-8.00 g/L	78	720	0-8.00 g/L	2350	720	N/A	---	---
Nickel, Heptoxime Method		0-1.80	335	430	0-1.80	32	430	0-1.80	2360	430	N/A	---	---
Nickel, PAN Method	yes	0-1.000	340	560	0-1.000	33	560	0-1.000	2370	560	0-1.000	48	560
Nitrate, High Range	yes	0-30.0	355	500	0-30.0	37	500	0-30.0	2530	500	0-30.0	51	520
Nitrate, High Range (AccuVac)	yes	0-30.0	361	500	0-30.0	88	500	0-30.0	2535	500	0-30.0	50	520
Nitrate, High Range, TNT		0-30.0	344	410	0-30.0	manual	410	0-30.0	2511	410	0-30.0	57	410
Nitrate, Low Range		0-0.40	351	507	0-0.50	39	507	0-0.50	2515	507	0-0.5	55	520
Nitrate, Mid Range		0-4.5	353	400	0-5.0	38	400	0-5.00	2520	400	0-5.00	54	420
Nitrate, Mid Range (AccuVac)		0-4.5	359	400	0-5.0	87	400	0-5.0	2525	400	0-5.00	53	420
Nitrate, Total, High range TNT		10-150	395	410	10-150	manual	410	10-150	2559	410	10-150	69	410
Nitrite, High Range		0-150	373	585	0-150	40	585	0-250	2600	585	0-150	59	560
Nitrite, Low Range		0-0.300	371	507	0-0.350	41	507	0-0.3000	2610	507	0-0.350	60	520
Nitrite, Low Range (AccuVac)		0-0.300	375	507	0-0.350	89	507	0-0.3000	2620	507	0-0.350	63	520

Tests Performed by Hach Photometers (Continued)

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Nitrite, Low Range TNT		0-0.50	345	507	0-0.50	manual	507	0-0.50	2558	507	0-0.50	58	507
Nitrogen, Ammonia, Nessler Method		0-2.50	380	425	0-3.00	34	425	0-2.50	2400	425	N/A	---	---
Nitrogen, Ammonia, Salicylate Method	yes	0-0.5	385	655	0-0.80	36	655	0.80	2455	655	0-0.5	64	610
Nitrogen, Ammonia, High Range, TNT		0-50.0	343	655	0-50.0	manual	655	0-50.0	2465	655	0-50.0	67	655
Nitrogen, Ammonia, Low Range, TNT,		0-2.50	342	655	0-2.50	manual	655	0-2.50	2460	655	0-2.50	66	655
Nitrogen, Total, TNT		0-25.0	350	410	0-25.0	manual	410	0-25.0	2558	410	0-25.0	58	410
Nitrogen, Total Inorganic, TNT		0-25.0	347	655	0-25.0	635	655	0-25.0	2550	655	0-25.0	68	655
Nitrogen, Total Kjeldahl (TKN)		0-150	399,9--	460	0-200	42	460	0-150	2410	460	0-159	65	420
Nitrogen, Monochloramine	yes	0-0.50	386	655	0-0.50	N/A	655	0-0.50	2470	655	0-0.50	49	610
Nitrogen, Monochloramine (AccuVac)		0-0.50	386	655	0-0.50	N/A	655	0-0.50	2470	655	0-0.50	49	610
Nitrogen, Free Ammonia	yes	0-0.50	386	655	0-0.50	N/A	655	0-0.50	2470	655	0-0.50	49	610
Nitrogen, Free Ammonia (AccuVac)		0-0.50	386	655	0-0.50	N/A	655	0-0.50	2470	655	0-0.550	49	610
Nitrogen, Nitrate, (direct reading)		N/A	---	---	N/A	---	---	0.0-10.2	2500	220	N/A	---	---
Organic Constituents		---	N/A	---	N/A	---	---	N/A	2460	253.7	N/A	---	---
Oxygen, Dissolved, High Range (AccuVac)	yes	0-13	445	535	0-15	104	535	0-15.0	2660	535	0-15.0	70	520

Tests Performed by Hach Photometers (Continued)

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Oxygen, Dissolved, Low Range (AccuVac)		0-800 µg/L	446	610	0-1000 µg/L	98	610	0-1000 µg/L	2650	610	0-1000 µg/L	71	610
Oxygen, Dissolved, Super High Range		0-45.0	448	680	N/A	---	---	0-40.0	2670	680	N/A	---	---
Oxygen Demand, Chemical, Dichromate Reflux Method		0-800	440	600	0-8000	92	600	N/A	---	---	N/A	---	---
Oxygen Demand, Chemical Reactor Digestion Method		N/A 0-150 0-1500 0-15,000	430 435 435	420 620 620	0-40 0-150 0-1500 0-15,000	103 45 46 46	350 420 620 620	0-40 0-150 0-1500 0-15,000	2700 2710 2720 2720	350 420 620 620	N/A 0-150 0-1500 0-15,000	16 17 17	420 610 610
Oxygen Demand, Chemical Manganese III		20-1000	432	510	20-1000	N/A	510	20-1000	2730	510	20-1000	18	420
Ozone, Indigo Method (AccuVac)	yes	0-0.25 0-0.75 0-1.50	454 455 456	600 600 600	0-0.25 0-0.75 0-1.50	95 96 97	600 600 600	0-0.25 0-0.75 0-1.50	2750 2760 2770	600 600 600	0-0.25 0-0.75 0-1.50	72 73 74	610 610 610
Palladium		0-250	460	420	N/A	---	---	0-250	2800	420	N/A		
PCB in Soil	yes	threshold	Abs mode	450	N/A	---	---	threshold	Abs	450	threshold	42	420
Phenols		0-0.200	470	460	0-0.200	48	460	0-0.200	2900	460	N/A	---	---
Phosphonates	yes	0-2.5 0-125	501	890	0-2.0 0-100	49	890	0-2.50 0-125	2950	890	0-2.5 0-125	80	610?
Phosphorus, Reactive, Amino Acid Method		0-30.00	485	530	0-20.00	50	530	0-30.00	3010	530	0-30.00	85	520
Phosphorus, Reactive, Molybdovanadate Method		0-45.0	480	430	0-20.00	75	400	0-45.0	3015	430	0-45.0	77	420
Phosphorus, Reactive, Molybdovanadate (AccuVac)		0-45.0	482	430	0-45.0	N/A	430	0-45.0	3020	430	0-45.0	78	420

Tests Performed by Hach Photometers (Continued)

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Phosphorus, Reactive, Molybdovanadate, TNT		0-100.0	540 541 for DR/2000	420	0-100.0	manual	420	0-100.0	3000	420	0-100.0	86	420
Phosphorus, Reactive PhosVer Method	yes	0-2.50	490	890	0-2.000	51	890	0-2.50	3025	890	0-2.50	79	610
Phosphorus, Reactive, PhosVer Method (AccuVac)	yes	0-2.50	492	890	0-2.000	91	890	0-2.50	3030	890	0-2.50	79	610
Phosphorus, Reactive, PhosVer Method, TNT		0.00-5.00	535	890	0.00-5.00	manual	890	0.00-5.00	3035	890	0.00-5.00	82	890
Phosphorus, Total, Test 'N Tube		0-3.50	535	890	0-3.50	N/A	890	0-3.50	3036	890	0-3.50	82	610
Phosphorus, Total, HR, Molybdovanadate Method		0-100.0	541	420	0-100.0	manual	420	0-100.0	3040	420	0-100.0	87	420
Phosphorus, Acid Hydrolyzable, TNT, PhosVer 3		0.00-5.00	535	890	0.00-5.00	manual	890	0.00-5.00	3037	890	0.00-5.00	82	890
Platinum		N/A	---	---	N/A	---	---	0-10 g/L	3100	500	N/A	---	---
Polyacrylic Acid: Accumer 1100 Accumer 1000		 0-20.0 0-20.0	 560 555	 482 482	 0-20.0 0-20.0	 555 556	 482 482	 0-20.0 0-20.0	 3050 3060	 482 482	N/A	---	---
Potassium		0-7.0	9--	650	N/A	---	---	0-7.0	N/A	650	N/A	---	---
Quaternary Ammonium Compound		0-5.0	401	575	0-5.0	106	575	0-5.00	3200	575	N/A	---	---
Residue, Nonfilterable		0-750	630	810	0-1000	52	810	N/A	---	---	0-750	94	610
Rhodium		---	N/A	---	N/A	---	---	N/A	---	---	N/A	---	---
Selenium		0-1.00	640	420	0-1.00	53	420	0-1.00	3300	420	N/A	---	---
Silica, High Range		0-100	656	452	0-30	54	410	0-100.0	3350	452	0-75	89	420
Silica, Low Range		0-1.600	651	815	0-1.600	55	815	0-1.600	3360	815	0-1.60	90	610

Tests Performed by Hach Photometers (Continued)

Test	Pocket Colorimeter	DR/2010, DR/2000 Spectrophotometers			DR/3000 Spectrophotometer			DR/4000 Spectrophotometer			DR/890 Colorimeter		
	Available	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)	Range (mg/L)	Program No.	Wave-length (nm)
Silica, Ultra Low Range		0-1000 µg/L	645	815	0-999.9 µg/L	N/A	815	0-1000 µg/L	3370	815	N/A	---	---
Silver		0-0.60	660	560	0-0.70	76	560	0-0.7	3400	560	N/A	---	---
Sodium Chromate		0-1100	670	460	0-1000	56	460	N/A	---	---	N/A	---	---
Sulfate		0-70	680, 9--	450	0-50	1	450	0-70.0	3450	450	0-70	91	520
Sulfate (AccuVac)		0-70	685, 9--	450	N/A	---	---	0-70.0	3460	450	0-70	92	520
Sulfide		0-0.600	690	665	0-0.800	57	665	0-800 µg/L	3500	665	0-0.700	93	610
Surfactants, Anionic (Detergents)		0-0.275	710	605	0-0.320	21	605	0-0.275	1850	605	0-0.300	26	610
Tannin and Lignin		0-9.0	720	700	0-9.00	58	700	0-9.0	3550	700	0-9.00	98	610
Tolyltriazole		0-16.0	730	425	0-20.0	4	425	0-16.0	3600	425	0-16.0	3	420
Toxicity		0-100%	Abs	603	0-100%	Abs	603	0-100%	Abs	603	0-100%	61	610
TPH in Water	yes	threshold	Abs mode	450	threshold	Abs	450	threshold	Abs	450	threshold	42	420
TPH in Soil	yes	threshold	Abs mode	450	threshold	Abs	450	threshold	Abs	450	threshold	42	420
Turbidity		0-450 FTU 0-4400 FAU	750 750	450[1] 860	0-600 FTU	59	450	0-5000 FAU	3750	860	0-1000 FAU	95	520
Volatile Acids		0-2800	770	495	0-2800	60	495	0-2800	3800	495	0-2800	96	520
Zinc	yes	0-2.00	780	620	0-2.500	61	620	0-3.000	3850	620	0-3.00	97	610

1 DR/2000 reads FTU at 450 nm; DR/2010 reads FAU at 860 nm.

Technical Training Workshops

Hach offers a variety of training workshops targeting a wide range of applications. These workshops help analysts from all backgrounds understand analytical theory, gain practical experience with the instrumentation and procedures, and return to their jobs with increased understanding and confidence.

Workshops, held in Loveland, Colorado, are small, personalized, and include lecture, classroom demonstration, and hands-on experience in the laboratory. Registration fees include all laboratory equipment, chemical reagents, and classroom materials used throughout the course of the workshop, as well as lunch and break refreshments. Attendance for workshops, most of which are two or three days in length, is limited and advanced registration is mandatory. For current workshop schedules, fees, and registration information, call 1-800-227-4224 extension 2391, fax 970-669-4807, or e-mail httc@hach.com.

Workshop contents are described below; please note they can be subject to change. Check our latest workshop information at our website, http:\\www.hach.com. Contact us, too, for information about customized training.

Basic Water Analysis/ Nonpotable Water
Reviews colorimetric technique for parameters such as nitrate, nitrite, ammonia, dissolved oxygen, low range phosphorus; titrimetric technique for dissolved oxygen, hardness, or alkalinity; and electrochemical theory for pH and conductivity measurement. Participants also apply sample digestion for total phosphate. Tests include use of standard solutions as well as actual samples and are completed with DR/2010 and DR/4000 Spectrophotometers, DR/800 Series Colorimeters, the Digesdahl Digestion Apparatus for rapid sample preparation, pH Meters and Electrodes, and Digital Titrators.

Basic Water Analysis/ Potable Water
Reviews colorimetric and electrochemical theory and techniques associated with analyzing general water quality. Participants will analyze standard solutions as well as real samples using DR/2010 and DR/4000 Spectrophotometers, DR/800 Series Colorimeters, turbidimeters, and pH/ISE Meters and Electrodes. Parameters measured: chlorine, nitrate, fluoride, pH, conductivity, and

turbidity. Methods for microbiological analysis will include rapid tests using Presence/Absence Tests, MPN and MF techniques.

Wastewater Analysis
Details methodologies for COD and BOD theory and measurement, electrochemical methods for pH and dissolved oxygen, digestion for total Kjeldahl nitrogen analysis using the Digesdahl® Digestion Apparatus, ToxTrak™ toxicity test demonstration, and colorimetric measurement of chlorine in the parts-per-billion range and different forms of nitrogen and phosphate. Tests include use of standard solutions as well as actual samples and are completed with DR/2010 and DR/4000 Spectrophotometers. Discussion also includes on-line measurement of some of these parameters for process control.

Boiler and Cooling Water Analysis
Reviews theory and techniques for colorimetric and electrochemical analysis of water quality in boilers and cooling towers as well as BART procedures for significant microorganisms. Participants conduct tests on standard solutions as well as actual samples using Hach DR/2010 and DR/4000 Spectrophotometers, pH Meters and Electrodes, and Digital Titrators. Parameters measured: alkalinity, chlorine, copper, hardness, iron, Langelier Index, phosphate, pH, silica, and molybdate. Some process analysis for control purposes is discussed.

Basic Water Analysis/Corrosivity
Reviews principles of testing for corrosion, including colorimetric measurement of iron, copper, chlorine, lead, dissolved oxygen—several methodologies are discussed and standard solution checks are included. Tests are completed with DR/2010 and DR/4000 Spectrophotometers and with colorimeters. Participants also discuss titrimetric measurement of hardness and alkalinity, electrochemical determination of pH and conductivity, the Langelier Index determination, and process analysis for control purposes. Instructors introduce color comparator discs, colorimeters, and spectrophotometers.

Microbiological Analysis
Review theory and proper enumeration, identification, and sampling techniques for microbiological testing. Participants will

analyze potable and nonpotable water samples for total coliform, fecal coliform and *E. coli* and evaluate and interpret results. Methods applied are: BARTS, Presence/Absence Tests, Paddle Testers, Heterotrophic Plate Count, MPN, and MF. Aseptic disposal technique and testing recreational waters also are discussed.

Science Educators Workshop
Offered during the summer months in Loveland, Colorado, public and private school educators review theory and techniques for water analysis, and receive guidelines for curriculum development appropriate for Grades 7-12. Workshop will cover colorimetric chemistry, titrations, and electrochemistry, along with guidelines on what to test, how to test, how the tests work, and how to interpret the results of the data.

Participants will conduct field testing of stream and pond water and will analyze standard solutions as well as real samples, using Hach test kits, the DR/2010 and DR/4000 Spectrophotometers, DR/800 Colorimeters, pH Meters and Electrodes, Digital Titrators, and comparator color wheels for easy, direct measurement.

Applied Quality Assurance/Quality Control with:
DR/4000 UV-VIS Spectrophotometer, or
DR/2010 Datalogging and DR/2000 Spectrophotometers
Topics include using the spectrophotometer diagnostics, keyboard functions, configuration, computer interface, and data storage. QA/QC and Lab Management topics such as data quality, accuracy, precision, method detection limits, and control charts are discussed and determined using the spectrophotometers. Participants also learn proper sample preparation and preservation and preparing calibration curves using prepared standard solutions.

Operation and Maintenance of:
1720 Series Low-Range Turbidimeter, or
CL17 Chlorine Analyzer, or
Series 5000 Silica Analyzer
These workshops detail installation, start-up and keyboard functions, calibration and routine maintenance, use of laboratory instruments for comparison, and computer interface using Aqua View+ Software.

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Discusses Hach's mission and goals, outlines a brief history and describes how to reach us through conventional means, such as telephone, fax and telex. Includes information about career opportunities and jobs with Hach (e-mail: **jobs@hach.com**).

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Describes workshops at the Hach Technical Training Center and provides the schedule of classes. You can register on-line or call 1-800-227-4224 extension 2391.

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General Information

Hach's free newsletter provides up-to-date information for the analyst. Published three times per year, *News & Notes for the Analyst* contains updates on federal regulations, in-depth discussions of chemical methods, application notes for instrument or test procedures, and a Hach product update.

Products for Analysis catalog describes laboratory, portable, and on-line instruments, including microbiological testing products, spectrophotometers, pH/ISE electrodes and meters, digestion apparatus, and test kits, as well as labware, chemicals and reagents. USA customers: request literature number 3226. Customers outside the United States: request literature 3307.

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