Selenium

DOC316.53.01129

Diaminobenzidine Method[1]

Method 8194

0.01 to 1.00 mg/L Se

Scope and application: For water and wastewater

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

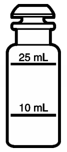
Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows sample cell and orientation requirements for reagent addition tests, such as powder pillow or bulk reagent tests.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information

Instrument	Sample cell orientation	Sample cell
DR 6000 DR 3800 DR 2800 DR 2700 DR 1900	The fill line is to the right.	2612602 
DR 5000 DR 3900	The fill line is toward the user.	

Before starting

A distillation is necessary to measure total selenium. Refer to Distillation on page 6. Use the distillate as the sample in step 3 of the test procedure.
Set up the distillation apparatus in a fume hood to prevent exposure to hazardous gas.
Use acetone to remove toluene from glassware after the measurements.
If there are visible water bubbles on the bottom of the prepared sample cell, decant the top portion into a clean, dry sample cell, then insert into the cell holder.
In bright light conditions (e.g., direct sunlight), close the cell compartment, if applicable, with the protective cover during measurements.
Do not pour toluene solutions down the drain. Collect the water saturated with toluene, toluene solutions and the cotton plug used in the delivery tube of the separatory funnel for proper disposal. Refer to a current MSDS/SDS for safe handling and disposal instructions.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

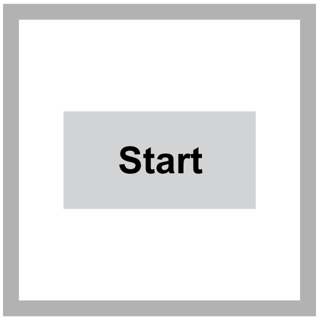
Description	Quantity
Buffer Solution, sulfate type, pH 2.0	10 mL
Distillation reagents and apparatus, refer to Consumables and replacement items on page 9	–
Cotton ball	1
Cylinder, Graduated: 50-mL and 100-mL	1 of each
Diaminobenzidine, tetrahydrochloride	0.1 g
Dropper, 0.5 and 1.0 mL marks, one glass and one plastic	1 of each
Flask, Erlenmeyer, 500-mL	2
Funnel, separatory, 250-mL	2
Hot Plate, 7-inch	1
Pipet, volumetric, 5-mL, plus safety bulb filler	1
Potassium Hydroxide Standard Solution, 12 N	4 mL
Ring support (3-inch) and stand	2
Sample cells (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	2
Spoons, measuring, 0.2 and 0.05 g	1 of each
TitraVer® Hardness Reagent	0.4 g
Toluene, ACS	60 mL
Water, deionized	100 mL

Refer to Consumables and replacement items on page 9 for order information.

Sample collection and storage

- Collect samples in clean glass or plastic bottles that have been cleaned with 6 N (1:1) hydrochloric acid and rinsed with deionized water.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with Nitric acid (approximately 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at room temperature for a maximum of 6 months.
- Before analysis, adjust the pH to 1-2 with 5 N Sodium Hydroxide Standard Solution.
- Correct the test result for the dilution caused by the volume additions.

Test procedure

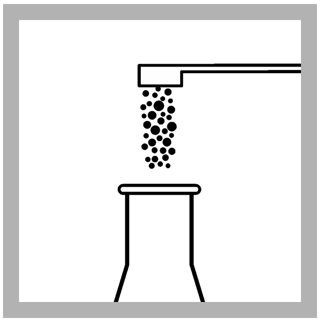


1. Start program **640 Selenium**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.

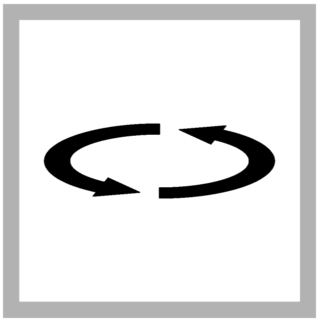
Note: *Although the program name can be different between instruments, the program number does not change.*

2. Prepare the blank: Use a graduated cylinder to add 100 mL of deionized water into a 500-mL Erlenmeyer flask. Put a mark on the flask to identify the flask as the blank.

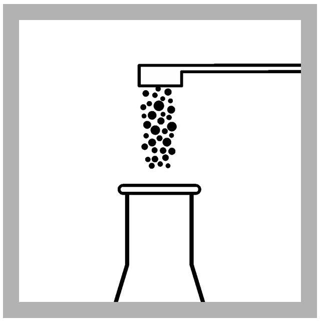
3. Prepare the sample: Use a graduated cylinder to add 100 mL of sample into a 500-mL Erlenmeyer flask. Put a mark on the flask to identify the flask as the sample.



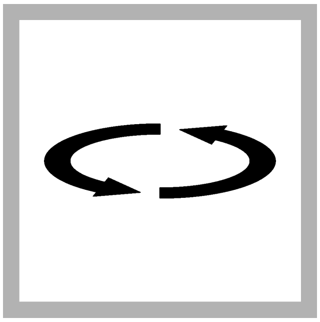
4. Add a 0.2-g spoonful of TitraVer Hardness Reagent to each flask.



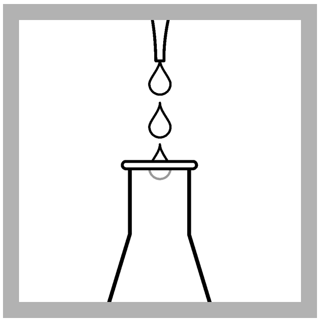
5. Swirl to mix.



6. Add a 0.05-g spoonful of diaminobenzidene tetrahydrochloride to each flask.

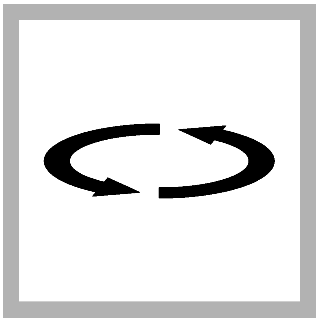


7. Swirl to mix.

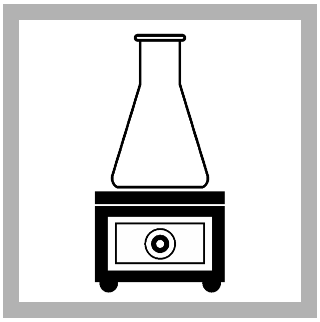


8. For non-distilled samples: Add 5.0 mL of Buffer Solution, sulfate type, pH 2.0 to each flask.

For distilled samples: Adjust the pH of the distillate to pH 2.7 ± 0.2 with 5 N Sodium Hydroxide Standard Solution. Adjust the pH of the blank to pH 2.7 ± 0.2 with 5.25 N Sulfuric Acid Standard Solution.



9. Swirl to mix.

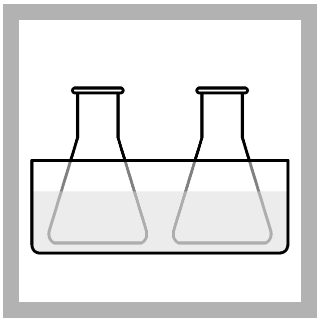


10. Put each flask on a hot plate and heat to a gentle boil.

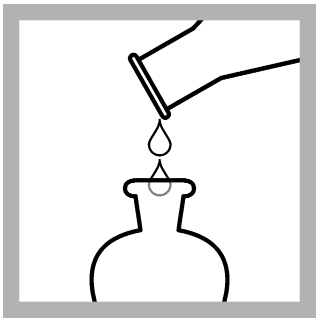
11. Start the instrument timer. A 5-minute reaction time starts.

Continue to carefully boil the contents during this time period. Do not boil more than 1 minute after the timer expires.

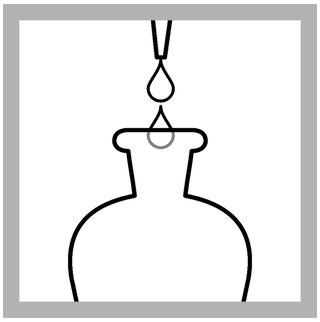
A yellow color forms if selenium is in the solution.



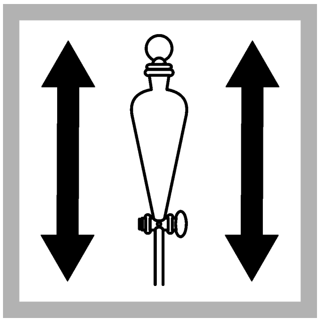
12. When the timer expires, remove both flasks from the hot plate. Decrease the temperature of the flasks to approximately 25 ºC (77 ºF) in a water bath.



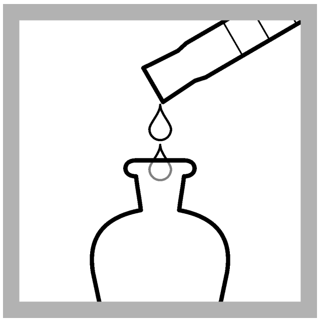
13. Pour the contents of each flask into separate 250-mL separatory funnels. Identify the funnels as "blank" and "sample". Place each funnel in a support ring on a stand.



14. Use a calibrated 1.0-mL plastic dropper to add 2.0 mL of 12 N Potassium Hydroxide Standard Solution to each funnel. Put the stopper on the funnels.

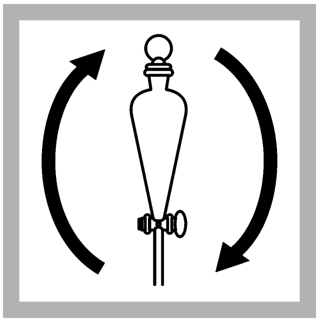


15. Shake each funnel to mix.



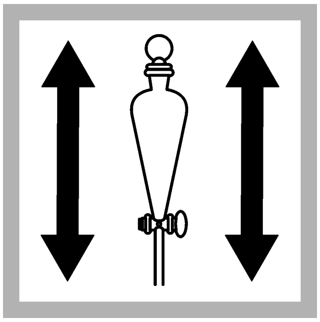
16. Add 30 mL of toluene to each funnel. Put the stopper on the funnels.

***Note: Gas inhalation hazard.** Use toluene only in a fume hood or with sufficient airflow to prevent exposure to hazardous gas.*



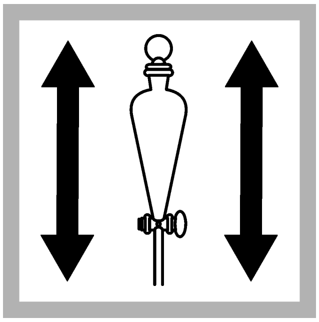
17. Swirl and invert each funnel, then open the stopcock to vent the funnel. Close the stopcock. Do this step two times with each funnel.

18. Start the instrument timer. A 30-second reaction time starts.



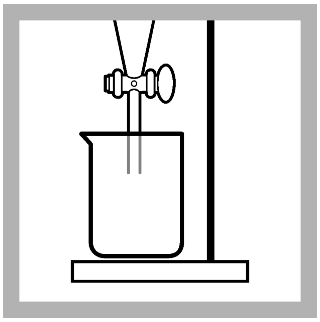
19. During this time, vigorously shake the funnel that contains the blank.

20. Start the instrument timer. A 30-second reaction time starts.

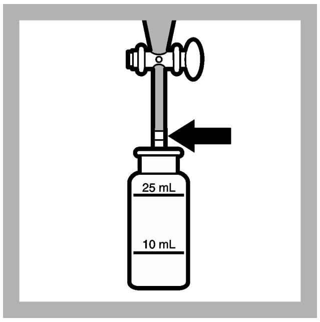


21. During this time, vigorously shake the funnel that contains the sample.

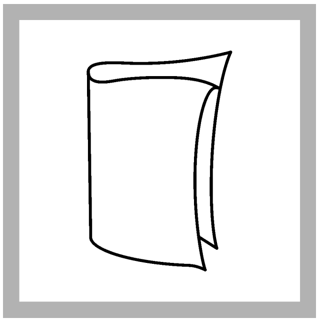
22. Start the instrument timer. A 4-minute reaction time starts.



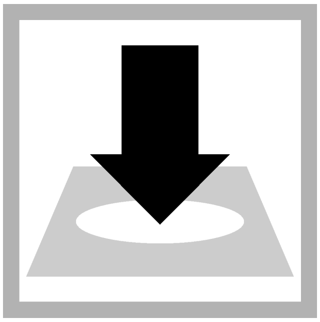
23. When the timer expires, remove the stopper and drain the lower water layer from each funnel and retain for disposal.
Complete steps 24–29 within 5 minutes after the timer expires. Measure the color as soon as possible.



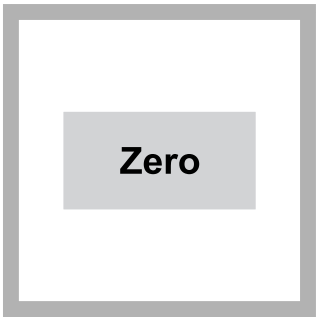
24. Insert a dry cotton plug into the outlet tube of each separatory funnel to filter the toluene and remove water or suspended particles. Identify the sample cells as blank and sample. Slowly drain the toluene from the funnels into the respective sample cells. Close the sample cells with stoppers.



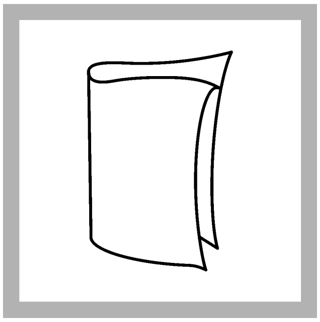
25. Clean the blank sample cell.



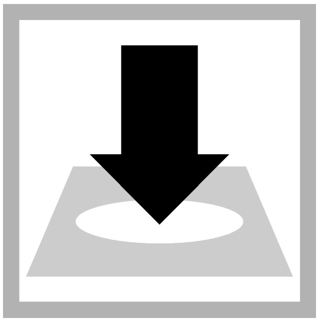
26. Insert the blank into the cell holder.



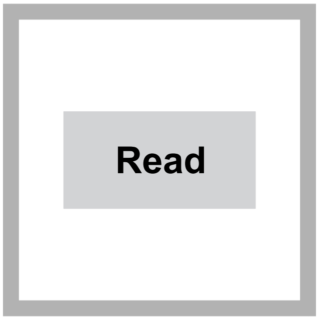
27. Push **ZERO**. The display shows 0.00 mg/L Se.



28. Clean the prepared sample cell.



29. Insert the prepared sample into the cell holder.



30. Push **READ**. Results show in mg/L Se.

Interferences

There are no positive inorganic interferences with this method. Any other interferences can be removed by distilling the sample. Refer to Table 2.

Table 2 Interfering substances

Interfering substance	Interference level
Ferric iron	Up to 2.5 mg/L. Distill sample to eliminate interference.
Manganese	Will not interfere.
Strong oxidizing agents (i.e., iodine, bromine or chlorine)	Can react with the indicator to give low results. Distill sample to eliminate interference.

Distillation

⚠ WARNING

Gas inhalation hazard. Operate the instrument in a fume hood to prevent exposure to hazardous gas.

The use of a strong acid and oxidizer at high temperatures is necessary for this distillation. To prevent personal injury, observe all laboratory safety precautions when operating the distilling apparatus.

To remove most interferences, distill the sample, then use the distilled sample in the test procedure.

Prepare the distillation solution:

1. Measure 500 mL of sample into a 1000-mL beaker.
2. Add 1 mL of Methyl Orange Indicator Solution. Stir with a glass rod.
3. Use a dropper to add 0.1 N Hydrochloric Acid Standard Solution, one drop at a time until the solution is pink. Then add 2 mL more of 0.1 N Hydrochloric Acid Standard Solution.
4. Use a pipet to add 5.0 mL Calcium Chloride Solution. Mix well.
5. Use a dropper to add 1-g/L Potassium Permanganate Standard Solution, one drop at a time until the solution is purple.
6. Put the beaker on a hot plate. Heat the solution until the volume is approximately 250 mL. As needed, add 1-g/L Potassium Permanganate Solution to keep the solution purple.
   Note: *Any precipitate formed at this step is manganese dioxide and can be ignored.*
7. Let the temperature of the solution decrease until room temperature.

Distillation procedure:

1. Set up the distillation apparatus for general purpose distillation. Refer to the Distillation Apparatus manual for proper assembly.
2. Pour the distillation solution into the distillation flask. Add a magnetic stir bar to the flask.
3. Use a pipet to add 5.0 mL of 0.1 N Sodium Hydroxide Standard Solution into the flask.
4. Set the stirrer power to on. Set the stir control to 5.
5. Turn on the water and adjust to maintain a steady flow through the condenser.
6. Set the heat control to 10.
7. When only a few milliliters are left in the distillation flask, set the heater power to off. Discard the distillate in the Erlenmeyer flask.
8. Let the temperature of the flask decrease to 20–25 ºC (68–77 ºF).
9. Add 50 mL of 19.2 N Sulfuric Acid Standard Solution to the flask.

10. Add 10 grams of Potassium Bromide to the flask.
11. Add 75 mL of deionized water to a 250-mL beaker. Place the beaker under the drip tube. Use a laboratory jack to elevate the beaker so the tube extends below the level of the water.
12. Add 1.0 mL of 30% hydrogen peroxide solution to the distillation flask. Set the stir control to 5 and the heat control to 10. Put the cap on the distillation flask.
13. Apply heat to the distillation flask until the yellow color is gone from the apparatus, including the J-tube and condenser. Remove the beaker from under the drip tube.
14. Set the heater power to off. Let the temperature of the J-tube and condenser decrease until room temperature. Rinse them with deionized water. Add the rinse water to the 250-mL beaker. The total volume in the beaker must be approximately 100 mL.
15. Add the Phenol Solution, 1 drop at a time, to the distilled sample to remove the bromine color (a white precipitate forms).
16. Let the precipitate settle. Use a dropper to collect approximately 5 mL of the clear, colorless distillate and add it to a test tube.
17. Add 2 drops of Phenol Solution to test for completeness of precipitation. If the solution contains residual bromide, a white turbidity or precipitate will form. Go to the next step. If there is no turbidity or precipitate, pour the 5 mL aliquot back into the beaker and go to step 19.
18. Pour the 5-mL aliquot back into the beaker. Continue to add Phenol Solution until there is no turbidity in subsequent 5-mL aliquots.
19. Pour the entire sample into a 500-mL volumetric flask. Rinse the beaker with deionized water and add the rinse water to the flask.
20. Dilute to the mark with deionized water. Put the stopper on the flask and mix well. The distillate is now ready for analysis.

Accuracy check

Standard additions method (sample spike)

Use the standard additions method (for applicable instruments) to validate the test procedure, reagents and instrument and to find if there is an interference in the sample.

Items to collect:

- 1000-mg/L Selenium Standard Solution
- 100-mL volumetric flask
- 10-mL volumetric pipet and pipet bulb
- Deionized water
- Pipet, TenSette®, 0.1–1.0 mL and tips

1. Prepare a 100-mg/L Selenium standard solution as follows:
   a. Use a pipet to add 10 mL of a 1000-mg/L selenium standard solution into a 100-mL volumetric flask.
   b. Dilute to the 100-mL mark with deionized water. Mix well. Prepare this solution daily.
2. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
3. Go to the Standard Additions option in the instrument menu.
4. Select the values for standard concentration, sample volume and spike volumes.
5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the prepared standard solution, respectively, to three 100-mL portions of fresh sample. Mix well.

6. Use the test procedure to measure the concentration of each of the spiked samples. Start with the smallest sample spike. Measure each of the spiked samples in the instrument.
7. Select **Graph** to compare the expected results to the actual results.

   Note: *If the actual results are significantly different from the expected results, make sure that the sample volumes and sample spikes are measured accurately. The sample volumes and sample spikes that are used should agree with the selections in the standard additions menu. If the results are not within acceptable limits, the sample may contain an interference.*

Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- 1000-mg/L Selenium Standard Solution
- 100-mL volumetric flask
- 200-mL volumetric flask
- Pipet, TenSette®, 1.0–10 mL and tips
- Deionized water

1. Prepare a 100-mg/L selenium stock solution as follows:
   a. Use a pipet to add 10 mL of a 1000-mg/L Selenium Standard Solution into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare the stock solution each day.
2. Prepare a 0.5-mg/L selenium standard solution as follows:
   a. Use a pipet to add 1.00 mL of the 100-mg/L selenium stock solution into a 200-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare the standard solution each day.
3. Use the test procedure to measure the concentration of the prepared standard solution.
4. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are slight variations in the reagents or instruments.*

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% confidence interval)	Sensitivity Concentration change per 0.010 Abs change
640	0.50 mg/L Se	0.47–0.53 mg/L Se	0.01 mg/L Se

Summary of Method

An EDTA masking agent is added to the sample to remove interferences such as iron. The addition of a sulfate buffer adjusts the sample to the optimum pH of 1 to 2. Under these conditions, diaminobenzidine reacts with selenite (Se^{4+}). The reaction gives a yellow piazselenol complex, which is extracted and the color intensity measured colorimetrically. Sample distillation is necessary to measure selenium as Se^{2+} or Se^{6+}. The measurement wavelength is 420 nm.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
Selenium Reagent Set (100 tests[1]), includes:			2244200
Buffer Solution, sulfate type, pH 2.0	10 mL	500 mL	45249
Diaminobenzidine, tetrahydrochloride	0.1 g	5 g	706222
Potassium Hydroxide Standard Solution, 12 N (x2)	4 mL	100 mL	23032
TitraVer® Hardness Reagent, ACS	0.4 g	100 g	20426
Toluene, ACS	60 mL	4 L	1447017
Water, deionized	varies	4 L	27256

[1] This test requires a reagent blank. The number of tests shown refers to any combination of samples and blanks.

Required apparatus

Description	Quantity/test	Unit	Item no.
Cotton Balls, absorbent	1	100/pkg	257201
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Dropper, measuring, 0.5-mL and 1.0-mL glass	1	5/pkg	1419705
Dropper, measuring, 0.5-mL and 1.0-mL plastic	2	20/pkg	2124720
Flask, Erlenmeyer, 500-mL	2	each	50549
Funnel, separatory, 250-mL	2	each	52046
Hot plate, 7 inch x 7 inch, digital, 120 VAC	1	each	2881500
Hot plate, stirrer, 220–240 VAC	1	each	2881602
Pipet, volumetric, Class A, 5.00-mL	1	each	1451537
Pipet filler, safety bulb	1	each	1465100
Ring, support, (3-inch) 83-mm	2	each	58000
Sample Cells, 1-inch square, 25 mL with stopper, matched pair	2	2/pkg	2612602
Spoon, measuring, 0.05-g	1	each	49200
Spoon, measuring, 0.2-g	1	each	63800
Support, Ring Stand, 5-inch x 8-inch base	1	each	56300

Distillation reagents and apparatus

Description	Unit	Item no.
Calcium Chloride Solution	1000 mL	42853
Hydrochloric Acid Standard Solution, 0.1 N	1000 mL	1481253
Hydrogen Peroxide, 30%, ACS	473 mL	14411
Methyl Orange Indicator Solution, (0.50-g/L)	500 mL	14849
Phenol Solution, 30-g/L	29 mL	211220
Potassium Permanganate Solution, 1-g/L	100 mL	1416442
Potassium Bromide, ACS grade[1]	–	–
Sodium Hydroxide Standard Solution, 0.100 N	1000 mL	19153

Distillation reagents and apparatus (continued)

Description	Unit	Item no.
Sulfuric Acid Standard Solution, 19.2 N	500 mL	203849
Distillation heater and support for apparatus set, 115 VAC option	each	2274400
Distillation heater and support for apparatus set, 230 VAC option	each	2274402
Distillation apparatus set, general purpose	each	2265300
Beaker, 1000-mL	each	5005
Glass stirring rod	3/pkg	177001
Stir bar, magnetic	each	2095351
Beaker, 250-mL	each	50046H
Test tubes, 24 mL, 16 mm diameter	10/pkg	56524

[1] Purchase Potassium Bromide from a local chemical supplier

Recommended standards

Description	Unit	Item no.
Selenium Standard Solution, 1000-mg/L	100 mL	2240742

Optional reagents and apparatus

Description	Unit	Item no.
Acetone, ACS	500 mL	1442949
Nitric Acid, concentrated	500 mL	15249
Sodium Hydroxide Standard Solution, 5.0 N	100 mL MDB	245032
Sulfuric Acid Standard Solution, 5.25 N	100 mL	244932
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Bottle, sampling, with cap, low density polyethylene, 250-mL	12/pkg	2087076
Paper, pH, 0–14 pH range	100/pkg	2601300
Flask, volumetric, Class A, 100-mL	each	1457442
Flask, volumetric, Class A, 200-mL	each	1457445
Pipet, volumetric, Class A, 10-mL	each	1451538
Pipet, volumetric, Class A, 1.00-mL	each	1451535

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