Dissolved Oxygen

Water Quality Test Kit

Instruction Manual
Code 5860-01

LaMotte

WARNING! This set contains chemicals that may be harmful if misued. Read cautions on individual containers carefully. Not to be used by children except under adult supervision.

INTRODUCTION

Aquatic animals need dissolved oxygen to live. Fish, invertebrates, plants, and aerobic bacteria all require oxygen for respiration. Oxygen dissolves readily into water from the atmosphere until the water is saturated. Once dissolved in the water, the oxygen diffuses very slowly and distribution depends on the movement of the aerated water. Oxygen is also produced by aquatic plants, algae, and phytoplankton as a by-product of photosynthesis.

This test kit uses the azide modification of the Winkler method for determining dissolved oxygen.

DISSOLVED OXYGEN, PERCENT SATURATION & BOD

Oxygen is critical to the survival of aquatic plants and animals, and a shortage of dissolved oxygen is not only a sign of pollution, it is harmful to fish. Some aquatic species are more sensitive to oxygen depletion than others, but some general guidelines to consider when analyzing test results are:

5–6 ppm	Sufficient for most species
<3 ppm	Stressful to most aquatic species
<2 ppm	Fatal to most species

Because of its importance to the fish's survival, aquaculturists, or "fish farmers," and aquarists use the dissolved oxygen test as a primary indicator of their system's ability to support healthy fish.

WHERE DOES THE OXYGEN COME FROM?

The oxygen found in water comes from many sources, but the largest source is oxygen absorbed from the atmosphere. Wave action and splashing allows more oxygen to be absorbed into the water. A second major source of oxygen is aquatic plants, including algae; during photosynthesis plants remove carbon dioxide from the water and replace it with oxygen.

Absorption

Oxygen is continuously moving between the water and surrounding air. The direction and speed of this movement is dependent upon the amount of contact between the air and water. A tumbling mountain stream or windswept, wave-covered lake, where more of the water's surface is exposed to the air, will absorb more oxygen from the atmosphere than a calm, smooth body of water. This is the idea behind aerators: by creating bubbles and waves the surface area is increased and more oxygen can enter the water.

Photosynthesis

In the leaves of plants, one of the most important chemical processes on Earth is constantly occurring: photosynthesis. During daylight, plants constantly take carbon dioxide from the air, and in the presence of water convert it to oxygen and carbohydrates, which are used to produce additional plant material. Since photosynthesis requires light, plants do not photosynthesize at night, so no oxygen is produced. Chemically, the photosynthesis reaction can be written as:

$$\text{Light} + nCO_2 + nH_2O \longrightarrow (C_2HO)n + nO_2$$

Light + Carbon Dioxide + Water ⟶ Carbohydrate + Oxygen

WHERE DOES THE OXYGEN GO?

Once in the water, oxygen is used by the aquatic life. Fish and other aquatic animals need oxygen to breathe or respire. Oxygen is also consumed by bacteria to decay, or decompose, dead plants and animals.

Respiration

All animals, whether on land or underwater, need oxygen to respire, grow and survive. Plants and animals respire throughout the night and day, consuming oxygen and producing carbon dioxide, which is then used by plants during photosynthesis.

Decomposition

All plant and animal waste eventually decomposes, whether it is from living animals or dead plants and animals. In the decomposition process, bacteria use oxygen to oxidize, or chemically alter, the material to break it down to its component parts. Some aquatic systems may undergo extreme amounts of oxidation, leaving no oxygen for the living organisms, which eventually leave or suffocate.

PERCENT SATURATION

The oxygen level of a water system is not only dependant on production and consumption. The potential dissolved oxygen capacity of water is limited by atmospheric pressure (altitude), salinity, and temperature. These factors determine the highest DO level possible. The percent saturation value expresses the quantity of dissolved oxygen in the sample as a percent of the theoretical potential.

When water holds all of the dissolved oxygen that it can hold at a given altitude, temperature, and salinity, it is said to be 100% saturated. If it holds a quarter as much as it could possibly hold under those conditions it is 25% saturated. It is possible to get percent saturation values over 100% when water becomes highly aerated by tumbling over rapids and dams. It can also become supersaturated on a sunny day when dense areas of plants or algae produce oxygen through photosynthesis.

Low atmospheric pressure found at higher altitudes slightly decreases the solubility of oxygen in water so the dissolved oxygen value must be corrected for altitude.

The various minerals dissolved in water lower the capacity of the water to hold oxygen. A correction factor can also be applied to dissolved oxygen measurements in saline waters. In fresh water, where the salinity is very low, this effect is insignificant when compared to the effect of temperature. Therefore, a correction for salinity is not incorporated into the calculation.

Cold water can hold more oxygen than warm water. That is why fish that require higher levels of oxygen, like trout, are found in cold water and dissolved oxygen concentrations are usually higher in the winter than they are in the summer at the same location. The percent saturation concentration can be corrected for water temperature.

Percent saturation levels from 80 to 120 percent are considered to be excellent. Levels between 60 and 79 percent are adequate. Above 125 percent and below 60 percent saturation, levels are poor. Fish and invertebrates that can move will leave areas with low dissolved oxygen and move to areas with higher levels. Slow moving, trapped or non-mobile aquatic animals may perish if levels become too low. Extremely high dissolved oxygen concentrations are harmful to fish even for very short periods of time. Gas bubble disease, which is characterized by the rupturing of capillaries in the gills due to supersaturated water, is usually fatal.

MEASURING BOD (BIOCHEMICAL OXYGEN DEMAND)

Biochemical oxygen demand is determined by measuring the dissolved oxygen concentration in a freshly collected water sample and comparing it to the dissolved oxygen level in a sample that was collected at the same time but incubated under specific conditions for a specific length of time. The difference between the two oxygen levels represents the amount of oxygen required for the decomposition of organic material and the oxidation of chemicals in the water during the storage period, a measurement known as the BOD.

Unpolluted, natural waters will have a BOD of 5 ppm or less. Raw sewage may have levels of 150 to 300 ppm. Wastewater treatment plants must reduce BOD to levels specified in their discharge permits, usually between 8 and 150 ppm BOD.

TESTING DISSOLVED OXYGEN

The first step in a DO titration is the addition of Manganous Sulfate Solution (4167) and Alkaline Potassium Iodide Azide Solution (7166). These reagents react to form a white precipitate, or floc, of manganous hydroxide, $Mn(OH)_2$. Chemically, this reaction can be written as:

$$MnSO_4 + 2KOH \longrightarrow Mn(OH)_2 + K_2SO_4$$

Manganous Sulfate + Potassium Hydroxide → Manganous Hydroxide + Potassium Sulfate

Immediately upon formation of the precipitate, the oxygen in the water oxidizes an equivalent amount of the manganous hydroxide to brown-colored manganic hydroxide. For every molecule of oxygen in the water, four molecules of manganous hydroxide are converted to manganic hydroxide. Chemically, this reaction can be written as:

$$4Mn(OH)_2 + O_2 + 2H_2O \longrightarrow 4Mn(OH)_3$$

Manganous Hydroxide + Oxygen + Water → Manganic Hydroxide

After the brown precipitate is formed, a strong acid, such as Sulfamic Acid Powder (6286) or Sulfuric Acid, 1:1 (6141) is added to the sample. The acid converts the manganic hydroxide to manganic sulfate. At this point the sample is considered "fixed" and concern for additional oxygen being introduced into the sample is reduced. Chemically, this reaction can be written as:

$$2Mn(OH)_3 + 3H_2SO_4 \longrightarrow Mn_2(SO_4)_3 + 6H_2O$$

Manganic Hydroxide + Sulfuric Acid → Manganic Sulfate + Water

Simultaneously, iodine from the potassium iodide in the Alkaline Potassium Iodide Azide Solution is oxidized by manganic sulfate, releasing free iodine into the water. Since the manganic sulfate for this reaction comes from the reaction between the manganous hydroxide and oxygen, the amount of iodine released is directly proportional to the amount of oxygen present in the original sample. The release of free iodine is indicated by the sample turning a yellow-brown color. Chemically, this reaction can be written as:

$$Mn_2(SO_4)_3 + 2KI \longrightarrow 2MnSO_4 + K_2SO_4 + I_2$$

Manganic Sulfate + Potassium Iodide → Manganous Sulfate + Potassium Sulfate + Iodine

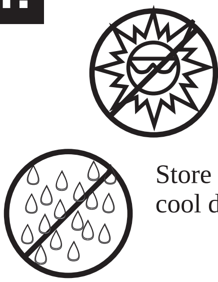
The final stage in the Winkler titration is the addition of sodium thiosulfate. The sodium thiosulfate reacts with the free iodine to produce sodium iodide. When all of the iodine has been converted the sample changes from yellow-brown to colorless. Often a starch indicator is added to enhance the final endpoint. Chemically, this reaction can be written as:

$$2Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$$

Sodium Thiosulfate + Iodine → Sodium Tetrathionate + Sodium Iodide

GENERAL SAFETY PRECAUTIONS

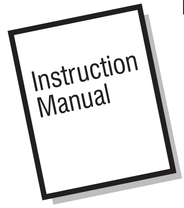
1.



Store the test kit in a cool dry area.

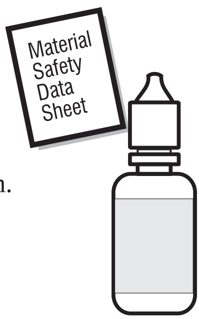
2.

Read all instructions and note precautions before performing the test procedure.



3.

Read the labels on all reagent bottles. Note warnings and first aid information. Read all Material Safety Data Sheets.



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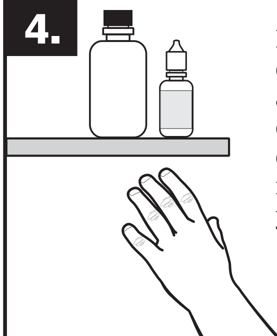


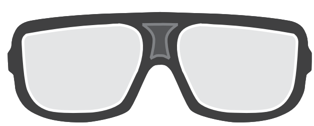
Keep all equipment and reagent chemicals out of the reach of young children.

5.

Avoid contact between reagent chemicals and skin, eyes, nose, and mouth.

6.

Wear safety glasses when performing test procedures.



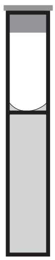
7.

In the event of an accident or suspected poisoning, immediately call the Poison Center phone number in the front of your local telephone directory or call a physician. Additional information for all LaMotte reagents is available in the United States, Canada, Puerto Rico, and the US Virgin Islands from Chem-Tel by calling 1-800-255-3924. For other areas, call 813-248-0585 collect to contact Chem-Tel's International access number. Each reagent can be identified by the four digit number listed on the upper left corner of the reagent label, in the contents list and in the test procedures.

USE PROPER ANALYTICAL TECHNIQUES

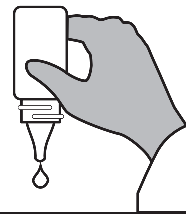
1.

Use test tube caps or stoppers, not your fingers, to cover tubes during shaking or mixing.



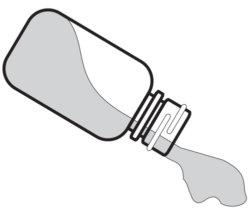
2.

Hold dropper bottles vertically upside-down, and not at an angle, when dispensing a reagent. Squeeze the bottle gently to dispense the reagent one drop at a time.



3.

Wipe up any reagent chemical spills immediately.



4.

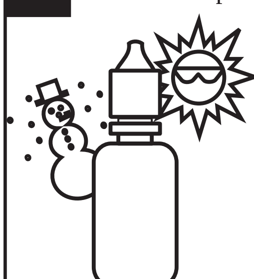
Thoroughly rinse test tubes before and after each test.

5.

Tightly close all containers immediately after use. Do not interchange caps from containers.

6.

Avoid prolonged exposure of equipment and reagents to direct sunlight. Protect reagents from extremes of temperature.



DISSOLVED OXYGEN

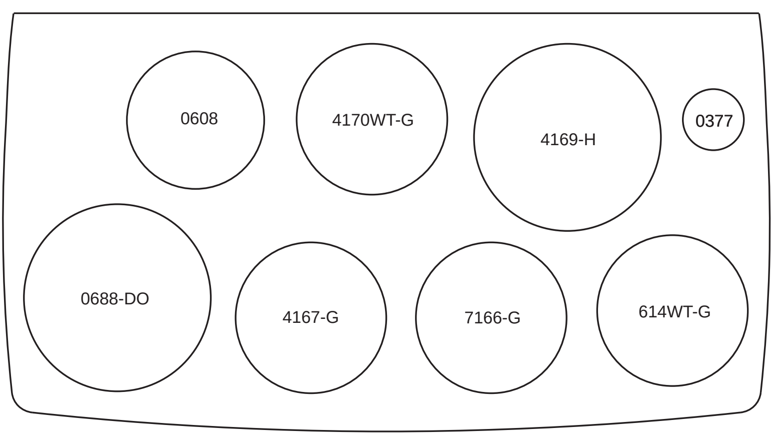
CODE 5860-01

QUANTITY	CONTENTS	CODE
30 mL	*Manganous Sulfate Solution	*4167-G
30 mL	*Alkaline Potassium Iodide Azide	*7166-G
30 mL	*Sulfuric Acid, 1:1	*6141WT-G
60 mL	*Sodium Thiosulfate, 0.025N	*4169-H
30 mL	Starch Indicator Solution	4170WT-G
1	Direct Reading Titrator	0377
1	Test Tube, 5-10-12.9-15-20-25 mL, glass, w/cap	0608
1	Water Sampling Bottle, 60 mL, glass	0688-DO

*WARNING: Reagents marked with an * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents go to www.lamotte.com. To obtain a printed copy, contact LaMotte by e-mail, phone or fax.

To order individual reagents or test kit components, use the specified code number.

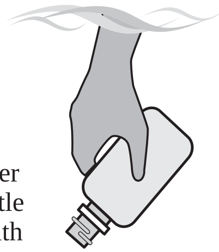
Kit Diagram



DISSOLVED OXYGEN TEST PROCEDURE

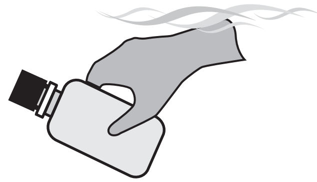
Part 1 - Collecting the Water Sample

1.



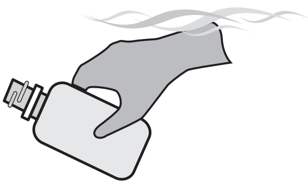
Rinse the Water Sampling Bottle (0688-DO) with the sample water.

2.



Tightly cap the bottle, and submerge it to the desired depth.

3.

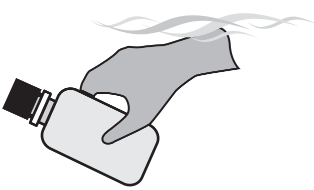


Remove the cap and allow the bottle to fill.

4.

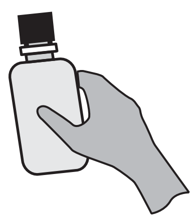
Tap the sides of the bottle to dislodge any air bubbles.

5.



Replace the cap while the bottle is still submerged.

6.



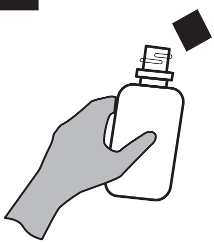
Retrieve the bottle and make sure that no air bubbles are trapped inside.

DISSOLVED OXYGEN TEST PROCEDURE

Part 2 - Adding the Reagents

NOTE: Be careful not to introduce air into the sample while adding the reagents.

1.



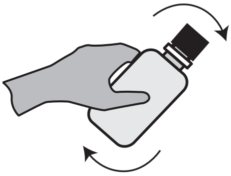
Remove the cap from the bottle.

2.

Immediately add 8 drops of *Manganous Sulfate Solution (4167) and Add 8 drops of *Alkaline Potassium Iodide Azide (7166).

3.

Cap the bottle and mix by inverting several times. A precipitate will form.



4.

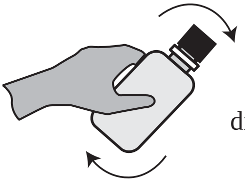
Allow the precipitate to settle below the shoulder of the bottle.

5.

Add 8 drops of *Sulfuric Acid, 1:1 (6141WT).

6.

Cap and gently invert the bottle to mix the contents until the precipitate and the reagent have totally dissolved. The solution will be clear yellow to orange if the sample contains dissolved oxygen.



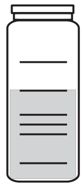
NOTE: At this point the sample has been “fixed” and contact between the sample and the atmosphere will not affect the test result. Samples may be held at this point and titrated later.

DISSOLVED OXYGEN TEST PROCEDURE

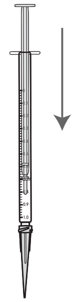
Part 3 - The Titration

1.

Fill the titration tube (0608) to the 20 mL line with the fixed sample. Cap the tube.



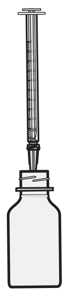
2.



Depress plunger of the Titrator (0377).

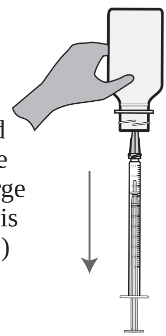
3.

Insert the Titrator into the plug in the top of the *Sodium Thiosulfate, 0.025N (4169) titrating solution.



4.

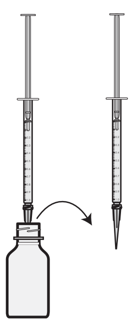
Invert the bottle and slowly withdraw the plunger until the large ring on the plunger is opposite the zero (0) line on the scale.

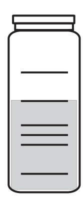


NOTE: If small air bubbles appear in the titrator barrel, expel them by partially filling the barrel and pumping the titration solution back into the reagent container. Repeat until bubble disappears.

5.

Turn the bottle upright and remove the Titrator.





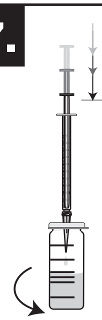
NOTE: If the sample is a very pale yellow, go to Step 9.

continued . . .

6.

Insert the tip of the Titraror into the opening of the titration tube cap.

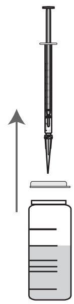
7.



Slowly depress the plunger to dispense the titrating solution until the yellow-brown color changes to a very pale yellow. Gently swirl the tube during the titration to mix the contents.

8.

Carefully remove the Titrator and cap. Do not disturb the Titrator plunger.

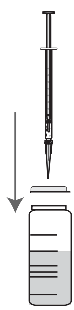


9.

Add 8 drops of Starch Indicator Solution (4170WT). The sample should turn blue.

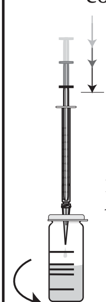
10.

Cap the titration tube. Insert the tip of the Titrator into the opening of the titration tube cap.



11.

Continue titrating until the blue color disappears and the solution becomes colorless.

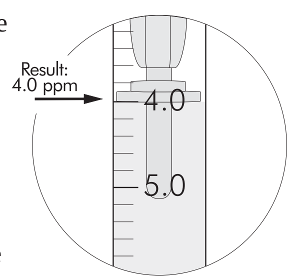


NOTE: If the plunger ring reaches the bottom line on the scale (10 ppm) before the endpoint color change occurs, refill the Titrator and continue the titration. Include the value of the original amount of reagent dispensed (10 ppm) when recording the test result.

12.

Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as ppm Dissolved Oxygen. Each minor division on the Titrator scale equals 0.2 ppm.

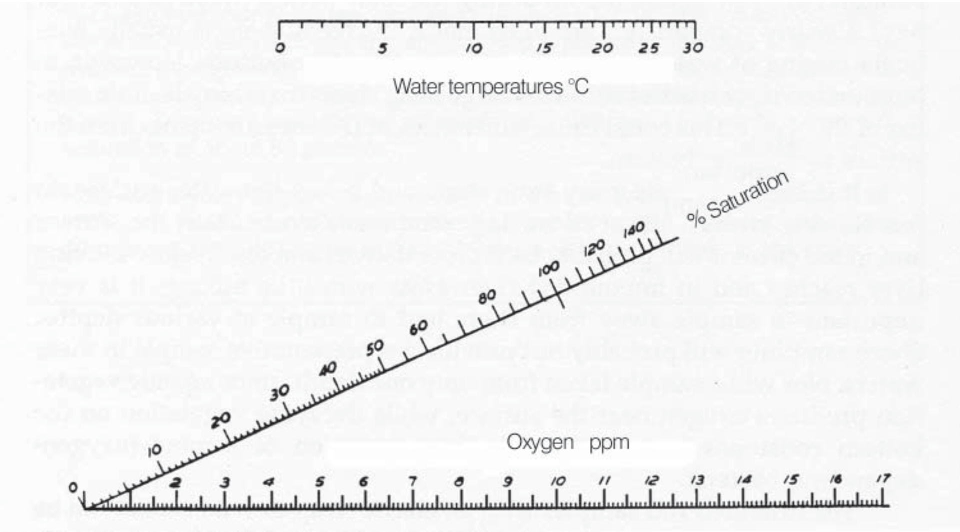
NOTE: When testing is complete, discard the titrating solution in the Titrator. Rinse Titrator and titration tube thoroughly. DO NOT remove plunger or adapter tip.



PERCENT SATURATION

Use the atmospheric pressure reading from a barometer or the local altitude to determine the correction factor from the chart below. Multiply the dissolved oxygen test result (ppm) by the correction factor to obtain the corrected dissolved oxygen value.

Atmospheric Pressure (mmHg)	Equivalent Altitude (ft)	Correction Factor
775	540	1.02
760	0	1.00
745	542	0.98
730	1094	0.96
714	1688	0.94
699	2274	0.92
684	2864	0.90
669	3466	0.88
654	4082	0.86
638	4756	0.84
623	5403	0.82
608	6065	0.80
593	6744	0.78
578	7440	0.76
562	8204	0.74
547	8939	0.72
532	9694	0.70
517	10,472	0.68

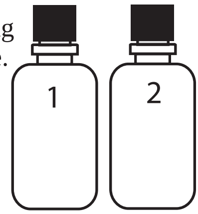


To determine the percent saturation, locate the temperature (°C) of the water sample on the top scale. Locate the corrected dissolved oxygen concentration (ppm) on the bottom scale. Draw a straight line between the two points. Read the % saturation where the line crosses the % saturation scale.

BOD

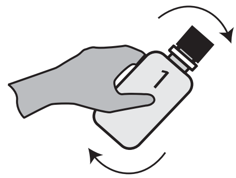
1.

Collect two samples according to Part 1 – Collecting the Water Sample.



2.

Test one sample immediately by following the procedures in Part 2 – Adding the Reagents and Part 3 – The Titration.



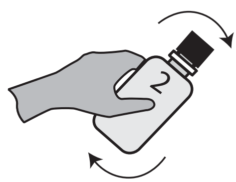
3.

Cover the bottle containing the second sample completely with aluminum foil to ensure complete darkness. This will prevent changes in the oxygen concentration caused by photosynthesis in algae that may be present in the sample.

4.

Incubate the second sample, holding the temperature at 20 °C for five days.

After five days, test the incubated sample by following the procedures in Part 2 – Adding the Reagents and Part 3 – The Titration.



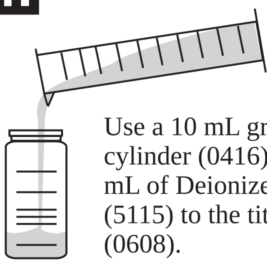
5.

Subtract the second dissolved oxygen reading from the initial dissolved oxygen reading to obtain BOD in units of ppm.

EPA COMPLIANCE

To qualify as an EPA accepted test, and to achieve the greatest accuracy, the Sodium Thiosulfate Solution, 0.025N (4169) must be standardized daily. This procedure follows Standard Methods for the Examination of Water and Wastewater. Numbers in ( ) are for LaMotte products. These products are not included in this kit but can be ordered from LaMotte Company by using the specified code number.

1.



Use a 10 mL graduated cylinder (0416) to add 15 mL of Deionized Water (5115) to the titration tube (0608).

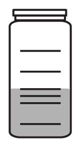
2.

Use a Direct Reading Titrator, 0-1 Range (1.0 mL capacity) (0376) to add 2 mL of Potassium Bi-iodate (7346).

3.

Add 2 drops of Sulfuric Acid, 5N (8517WT).

4.

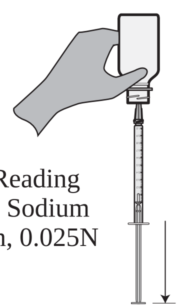


Use the 0.1 g spoon (0699) to add 0.2 g Potassium Iodide Crystals (6809).

5.

Swirl to dissolve. Solution will turn yellowish brown.

6.

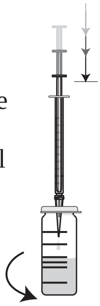


Fill another Direct Reading Titrator (0376) with Sodium Thiosulfate Solution, 0.025N (4169).

continued . . .

7.

While gently swirling the tube, add Sodium Thiosulfate, 0.025N until the color fades to pale yellow. It will be necessary to refill the Direct Reading Titrator.

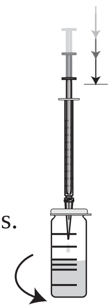


8.

Add 3 drops of Starch Indicator Solution (4170WT). The solution will turn blue.

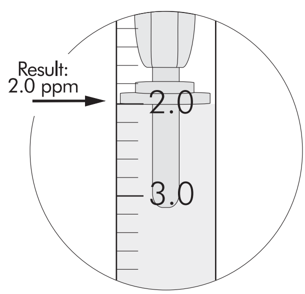
9.

Continue adding Sodium Thiosulfate, 0.025N until the blue color disappears and the solution is colorless.



10.

Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Include the value of the original amount dispensed (1 mL). If the reading is 2.0 +/- 0.1 mL, the Sodium Thiosulfate, 0.025N (4169) is satisfactory. If not, discard and replace with new reagent



SHORT FORM INSTRUCTIONS

Read all instructions before performing test. Use this guide as a quick reference.

1. Fill Water Sampling Bottle (0688-DO).
2. Add 8 drops of *Manganous Sulfate Solution (4167).
3. Add 8 drops of *Alkaline Potassium Iodide Azide (7166).
4. Cap and mix.
5. Allow precipitate to settle.
6. Add 8 drops of Sulfuric Acid, 1:1 (6141WT).
7. Cap and mix until reagent and precipitate dissolve.
8. Fill test tube (0608) to the 20 mL line.
9. Fill Titrator with *Sodium Thiosulfate, 0.025N (4169).
10. Titrate until sample color is pale yellow. DO NOT DISTURB TITRATOR.
11. Add 8 drops of Starch Indicator (4170WT).
12. Continue titration until blue color just disappears and solution is colorless.
13. Read result in ppm Dissolved Oxygen.

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