Fresh Water Aquaculture

Test Kit Instruction Manual
Code 3633-05
Warning! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision.
This booklet provides step-by-step detailed instructions for the Code 3633-05 test kits. It is important to review these instructions thoroughly before attempting to perform the tests by the short-form instructions contained in the case lid. To order individual reagents or test kit components, use the specified code number.

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Wipe up any reagent chemical spills, liquid or powder, as soon as they occur. Refer to label and accompanying SDS at www.lamotte.com for proper reagent disposal.

Avoid prolonged exposure of equipment and reagents to direct sunlight. Protect reagents and components from extreme heat and cold.

Tightly close all reagent containers immediately after use. Be sure not to interchange caps and pipets from different containers.

Use care when dispensing or handling all reagents. Some reagents also may cause permanent stains if spilled.
ANALYTICAL TECHNIQUE

1. Clean glassware is a must for accurate results. Thoroughly rinse test tubes before and after each test. Caps and stoppers should also be cleaned after each use.

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

3. When adding sample to calibrated test tube, be sure vial is filled to the appropriate mark. The bottom of the liquid (meniscus) should be level with the desired mark. (Fig. 1)

4. When dispensing reagents from bottles filled with dropper plug and cap, be sure to hold bottle vertically and gently squeeze to dispense the appropriate number of uniform drops. (Fig. 2)

5. For those reagents to be added with the screwcap pipet assemblies enclosed, remove polyseal cap on bottle and replace with the screwcap pipet. NOTE: Place the polyseal caps back on the reagent bottles for longer periods of storage. Be sure that both pipet assemblies and polyseal caps are thoroughly cleaned before placing on bottles to avoid contamination.

6. When dispensing reagents from pipets, hold pipet vertically to assure uniform drop size. This is extremely important when performing drop count titrations. (Fig. 3)

7. To fill pipets, squeeze rubber bulb and immerse into reagent. Release bulb to fill. (Fig. 4)

8. To accurately dispense powdered reagents with spoon, tap spoon on edge of reagent container to remove excess reagent. (Fig. 5)

9. When performing tests that include Octa-Slide Comparators, the comparator should be positioned between the operator and non-direct sunlight. This allows the light to enter through the light-diffusing screen at the back of the comparator for optimum color comparison.
GENERAL SAFETY PRECAUTIONS

Store the test kit in a cool dry area.

Read all instructions and note precautions before performing the test procedure. Read all Safety Data Sheets (SDS) at www.lamotte.com.

Read the labels on all reagent bottles. Note warnings and first aid information. Reagents marked with a * on instructions are considered possible health hazards.

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

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

Wear safety glasses when performing test procedures.

*WARNING: Reagents marked with an * are considered to be potential health hazards. To view or print a Safety Data Sheet (SDS) for these reagents go to www.lamotte.com. Search the four digit reagent code number listed on the reagent label, in the contents list or in the test procedures. Omit any letter that follows or precedes the four digit code number. For example, if the code is 4450WT-H, search 4450. To obtain a printed copy, contact LaMotte by e-mail, phone or fax.

Emergency information for all LaMotte reagents is available from Chem-Tel (US, 1-800-255-3924) (International, call collect, 813-248-0585).
TEST METHODS

This test kit uses two basic analytical procedures common to field test kits. A brief explanation of each follows:

COLORIMETRIC: OCTA-SLIDE 2 VIEWER

In a visual colorimetric test, a sample is treated with reagent(s) to produce a color reaction, generally in proportion to the amount of test factor present. The sample color is then compared against color standards representing known concentrations of the factor being tested over a specific range.

1. Insert Octa-Slide 2 Bar into the Octa-Slide 2 Viewer (1101).
2. Insert test tube containing reacted sample into the Octa-Slide 2 Viewer (1101).
3. Hold the Octa-Slide 2 Viewer so that non-direct light enters through the back of the viewer.

Note:
If sample color is between two standards, the midpoint is taken as the result.
If the sample is darker than the highest standard, a dilution may be performed on a fresh sample, and the test repeated to bring the concentration within range.

DILUTIONS

The calibrated test tubes (0106) included in this kit may be used to perform dilutions for the Ammonia Nitrogen and Nitrite Nitrogen tests. Distilled or deionized water is needed to perform dilutions.
The following table provides a quick reference guide for dilutions of various proportions. Once the dilution is prepared, use this diluted sample to perform the test, and multiply the result by the dilution factor to obtain the actual concentration.

<table>
<thead>
<tr>
<th>Sample Size</th>
<th>Distilled Water to Bring to 10 mL</th>
<th>Dilution Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 mL</td>
<td>5.0 mL</td>
<td>2</td>
</tr>
<tr>
<td>2.5 mL</td>
<td>7.5 mL</td>
<td>4</td>
</tr>
</tbody>
</table>
TITRIMETRIC: DIRECT READING TITRATOR

In a titrimetric method, titrating solution (or titrant) is added to a treated sample until a color change occurs. The volume of titrant required to reach this endpoint is proportional to the concentration of the factor being tested. Direct Reading Titrators provide results directly in the appropriate concentration for the test – no counting of drops, no calculations.

The Titrator consists of a plastic barrel, a plastic plunger, and a plastic adapter tip. The adapter tip reduces the size of the drops that are dispensed, increasing the precision of the test results. DO NOT remove the plunger or adapter tip from the Titrator.

1 Fill the test tube to the appropriate line with sample water.

2 Add reagents as specified in the instructions for the individual test method. Cap with the special test tube cap. Mix by swirling gently.

3 Depress the Titrator plunger to expel air. Insert Titrator into the plastic fitting of the titrating solution bottle and invert.

4 To fill Titrator, slowly withdraw the plunger until the bottom of the plunger is opposite the zero mark on the scale.

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

6 Turn the bottle right-side-up and remove the Titrator.

7 Insert the Titrator into the center hole of the test tube cap. While gently swirling tube, add titrating solution one drop at a time until the desired color changes occur. Follow individual test instructions.

8 Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel.

9 When testing is complete, discard titrating solution in Titrator. Do not return the titrant to the reagent bottle. Rinse Titrator and titration tube thoroughly. Do not remove the plunger or the adapter tip from the Titrator.
TEST PROCEDURES

INTRODUCTION

Proper control of water quality is an essential part of successful aquaculture operation. Immediate test results provided by on-site water analysis equipment can confirm a healthy environment, or give early warning signals for required treatment.

1. Develop a routine testing schedule.
2. Keep records! Historical data is extremely important if treatments are required. Note environmental conditions, fish activity, feeding habits, etc.
3. Observe fish to note any particular behavior or feeding rates, as this may be a sign of stress.
4. Stable characteristics, such as alkalinity and hardness, do not have to be tested as frequently as ones that fluctuate, such as ammonia nitrogen, nitrite nitrogen, pH, dissolved oxygen and temperature. Keep in mind that these factors fluctuate throughout the day and in some cases are interdependent.
5. Be alert to sudden changes in one factor, as it may be a clue to perform further analysis.
Alkalinity

**DESCRIPTION**

<table>
<thead>
<tr>
<th>CODE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>2311-PG-E</td>
<td>BCG/MR Indicator</td>
</tr>
<tr>
<td>*4493DR-H</td>
<td>*Alkalinity Titration Reagent B</td>
</tr>
<tr>
<td>0608</td>
<td>Test Tube, 5-10-12.9-15-20-25 mL, glass, w/cap</td>
</tr>
<tr>
<td>0382</td>
<td>Direct Reading Titrator, 0-200 Range</td>
</tr>
</tbody>
</table>

*WARNING: Reagents marked with an * are considered to be potential health hazards. See page 6 for more information.

The Direct Reading Titrator is calibrated in terms of total alkalinity expressed as parts per million (ppm) Calcium Carbonate (CaCO₃). Each minor division on the Titrator scale equals 4 ppm CaCO₃.

**ALKALINITY TEST PROCEDURE**

1. Fill the test tube (0608) to the 5 mL line with the sample water.


3. Cap and mix. Solution will turn blue-green.

4. Fill Direct Reading Titrator (0382) with *Alkalinity Titration Reagent B (4493DR).

5. Insert the Titrator into the center hole of the test tube cap.

6. While gently swirling the tube, slowly press the plunger to titrate until blue-green color changes to pink.

7. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record Total Alkalinity as ppm Calcium Carbonate (CaCO₃).

**Note:**

If the plunger tip reaches the bottom line on the scale (200 ppm) before the endpoint color change occurs, refill the Titrator and continue the titration.

When recording the test result, be sure to include the value of the original amount of reagent dispensed (200 ppm).
# Ammonia Nitrogen

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Salicylate Ammonia #1</td>
<td>*3978LWT-G</td>
</tr>
<tr>
<td>*Salicylate Ammonia #2</td>
<td>*3979WT-G</td>
</tr>
<tr>
<td>Salicylate Ammonia #3</td>
<td>3982WT-G</td>
</tr>
<tr>
<td>Test Tube, 2.5-5-10 mL, plastic, w/cap</td>
<td>0106</td>
</tr>
<tr>
<td>Octa-Slide 2 Viewer</td>
<td>1101</td>
</tr>
<tr>
<td>Ammonia Nitrogen Octa-Slide 2 Bar, 0-2.0 ppm, Fresh Water</td>
<td>3441-01-FW</td>
</tr>
</tbody>
</table>

*WARNING: Reagents marked with an * are considered to be potential health hazards. See page 6 for more information.*
AMMONIA NITROGEN TEST PROCEDURE

1. Insert Ammonia Nitrogen Octa-Slide 2 Bar (3441-01-FW) into the Octa-Slide 2 Viewer (1101).

2. Fill a test tube (0106) to the 5 mL line with the water sample.

3. Add 10 drops of *Salicylate Ammonia #1 (3978WT).

4. Cap and mix.

5. Add 7 drops of *Salicylate Ammonia #2 (3979WT).

6. Cap and mix.

7. Wait 1 minute.

8. Add 7 drops of Salicylate Ammonia #3 (3982WT).

9. Cap and mix.

10. Wait 20 minutes.

11. Insert test tube into Octa-Slide 2 Viewer.

12. Match sample color to a color standard. Record as ppm Ammonia Nitrogen.

Calculations:

To express results as Ammonia (NH₃):

\[
\text{Ammonia (NH₃)} = \text{ppm Ammonia Nitrogen (NH₃-N)} \times 1.2
\]

To express results as Ammonium (NH₄⁺):

\[
\text{Ammonium (NH₄⁺)} = \text{ppm Ammonia Nitrogen (NH₃-N)} \times 1.3
\]
AMMONIA IN AQUARIUMS

Ammonia in water occurs in two forms: toxic unionized ammonia \((\text{NH}_3)\) and the relatively non-toxic form, ammonium ion \((\text{NH}_4^+)\). This test method measures both forms as ammonia-nitrogen \((\text{NH}_3\text{-N})\) to give the total ammonia-nitrogen concentration in water. The actual proportion of each compound depends on temperature, salinity, and pH. A greater concentration of unionized ammonia is present when the pH value and salinity increase.

1. Consult the table below to find the percentage that corresponds to the temperature, pH and salinity of the sample.

2. To express the test result as ppm Unionized Ammonia Nitrogen \((\text{NH}_3\text{-N})\), multiply the total ammonia-nitrogen test result by the percentage from the table.

3. To express the test result as ppm Ionized Ammonia Nitrogen \((\text{NH}_4\text{-N})\), subtract the unionized ammonia nitrogen, determined in Step 2, from the total ammonia-nitrogen.

### Percentage of Free Ammonia as \([\text{NH}_3]\) in Freshwater\(^1\) (FW) and Seawater\(^2\) (SW) at varying pH and temperature.

<table>
<thead>
<tr>
<th>pH</th>
<th>10°C FW1</th>
<th>15°C FW</th>
<th>20°C FW</th>
<th>25°C FW</th>
<th>10°C SW2</th>
<th>15°C SW</th>
<th>20°C SW</th>
<th>25°C SW</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>0.19</td>
<td>0.27</td>
<td>0.40</td>
<td>0.55</td>
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<tr>
<td>7.1</td>
<td>0.23</td>
<td>0.34</td>
<td>0.50</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7.2</td>
<td>0.29</td>
<td>0.43</td>
<td>0.63</td>
<td>0.88</td>
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<tr>
<td>7.3</td>
<td>0.37</td>
<td>0.54</td>
<td>0.79</td>
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<td>7.4</td>
<td>0.47</td>
<td>0.68</td>
<td>0.99</td>
<td>1.38</td>
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<td>7.5</td>
<td>0.59</td>
<td>0.459</td>
<td>0.85</td>
<td>0.665</td>
<td>1.24</td>
<td>0.963</td>
<td>1.73</td>
<td>1.39</td>
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<td>7.6</td>
<td>0.74</td>
<td>0.577</td>
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<td>0.836</td>
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<td>1.21</td>
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<td>1.05</td>
<td>1.96</td>
<td>1.52</td>
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<td>2.19</td>
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<td>7.8</td>
<td>1.16</td>
<td>0.912</td>
<td>1.69</td>
<td>1.32</td>
<td>2.45</td>
<td>1.90</td>
<td>3.39</td>
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<td>7.9</td>
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<td>1.15</td>
<td>2.12</td>
<td>1.66</td>
<td>3.06</td>
<td>2.39</td>
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<td>8.0</td>
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<td>1.44</td>
<td>2.65</td>
<td>2.07</td>
<td>3.83</td>
<td>2.98</td>
<td>5.28</td>
<td>4.28</td>
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<tr>
<td>8.1</td>
<td>2.29</td>
<td>1.80</td>
<td>3.32</td>
<td>2.60</td>
<td>4.77</td>
<td>3.73</td>
<td>6.55</td>
<td>5.32</td>
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<td>8.2</td>
<td>2.86</td>
<td>2.26</td>
<td>4.14</td>
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<td>5.94</td>
<td>4.65</td>
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<td>6.61</td>
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<td>8.3</td>
<td>3.58</td>
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<td>3.54</td>
<td>6.41</td>
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<td>9.09</td>
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<tr>
<td>8.5</td>
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<td>4.41</td>
<td>7.98</td>
<td>6.28</td>
<td>11.18</td>
<td>8.87</td>
<td>14.97</td>
<td>12.40</td>
</tr>
</tbody>
</table>

\(^1\)Freshwater data from Trussel (1972).
\(^2\)Seawater values from Bower and Bidwell (1978). Salinity for Seawater values = 34 ppt at an ionic strength of 0.701 m.

FOR EXAMPLE:

A fresh water sample at 20°C has a pH of 8.5 and the test result is 1.0 ppm as total Ammonia-Nitrogen.

1. The percentage from the table is 11.18% (or 0.1118).

2. 1 ppm total Ammonia-Nitrogen x 0.1118 = 0.1118 ppm Unionized Ammonia-Nitrogen

3. Total Ammonia-Nitrogen - Unionized Ammonia-Nitrogen = Ionized Ammonia-Nitrogen

\[
\begin{align*}
\text{Total Ammonia-Nitrogen} & = 1.0000 \text{ ppm} \\
\text{Unionized Ammonia-Nitrogen} & = 0.1118 \text{ ppm} \\
\text{Ionized Ammonia-Nitrogen} & = 0.8882 \text{ ppm}
\end{align*}
\]
CARBON DIOXIDE TEST PROCEDURE

1. Fill the test tube (0608) to the 20 mL line with the sample water.

2. For best results, test a freshly obtained sample, and avoid splashing or prolonged contact with air.

3. Add 2 drops of *Phenolphthalein Indicator, 1% (2246). If sample turns red, no free carbon dioxide is present. If sample is colorless, proceed to Step 3.

4. Fill Direct Reading Titrator (0380) with Carbon Dioxide Reagent B (4253DR).

5. Insert the Titrator into the center hole of the test tube cap.

6. While gently swirling the tube, slowly press the plunger to titrate until a faint pink color develops and persists for 30 seconds.

7. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as Carbon Dioxide.

Note:
The Titrator is calibrated in terms of carbon dioxide expressed as ppm Free CO₂. Each minor division on the Titrator scale equals 1.0 ppm CO₂.
**Chloride**

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Chloride Reagent #1</td>
<td>*4504-E</td>
</tr>
<tr>
<td>*Chloride Reagent #2</td>
<td>*4505DR-H</td>
</tr>
<tr>
<td>*Phenolphthalein Indicator, 1%</td>
<td>*2246-E</td>
</tr>
<tr>
<td>*Sulfuric Acid, 0.5N</td>
<td>*6090-E</td>
</tr>
<tr>
<td>Direct Reading Titrator, 0-200 Range</td>
<td>0382</td>
</tr>
<tr>
<td>Test Tube, 5-10-12.9-15-20-25 mL, glass, w/cap</td>
<td>0608</td>
</tr>
</tbody>
</table>

*WARNING: Reagents marked with an * are considered to be potential health hazards. See page 6 for more information.

The Titrator is calibrated in terms of chloride expressed as ppm Cl\(^-\). Each minor division on the Titrator scale equals 4.0 ppm Cl\(^-\).

**HIGH CHLORIDE AND SALINITY READINGS**

For high chloride and salinity readings the sample must be carefully diluted to bring it within a practical range for titration. Dilutions of 1 to 20 or 1 to 100 are recommended. (For example: 1 mL of sample water is diluted to a total of 20 mL with distilled water. This is a 1 to 20 dilution.) Titration tube is then filled to 15 mL line with diluted sample, and the titration is performed as described. The Titrator reading is multiplied by the appropriate conversion factor given below for parts per million (ppm), parts per thousand (ppt), or percent (%) Chloride.

### 1 to 20 DILUTION

\[
\text{ppm chloride} = \text{Titrator Reading} \times 20 \\
\text{ppt chloride} = \text{Titrator Reading} \times 0.02 \\
\text{% chloride} = \text{Titrator Reading} \times 0.002
\]

### 1 to 100 DILUTION

\[
\text{ppm chloride} = \text{Titrator Reading} \times 100 \\
\text{ppt chloride} = \text{Titrator Reading} \times 0.1 \\
\text{% chloride} = \text{Titrator Reading} \times 0.01
\]

To convert parts per thousand (ppt) Chloride to parts per thousand (ppt) Salinity use the following formula:

\[
\text{ppt salinity} = (1.805 \times \text{ppt chloride}) + 0.03
\]
# Chloride Test Procedure

1. Fill the test tube (0608) to the 15 mL line with the sample water.

2. Add one drop of *Phenolphthalein Indicator, 1% (2246). If sample turns pink, add *Sulfuric Acid, 0.5N (6090) one drop at a time, mixing after each drop, until pink color disappears.

3. Add 3 drops of *Chloride Reagent #1 (4504).

4. Cap and swirl to mix. Solution will turn yellow.

5. Fill Direct Reading Titrator (0382) with *Chloride Reagent #2 (4505DR).

6. Insert the Titrator into the center hole of the test tube cap.

7. While gently swirling the tube, slowly press the plunger to titrate until yellow color changes from yellow to orange or orange-red.

8. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as ppm Chloride.

**Note:**

If the plunger tip reaches the bottom line on the scale (200 ppm) before the endpoint color change occurs, refill the Titrator and continue the titration. When recording the test results be sure to include the value of the original amount of reagent dispensed (200 ppm).
### Dissolved Oxygen

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>CODE</th>
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</thead>
<tbody>
<tr>
<td>*Manganous Sulfate Solution</td>
<td>*4167-G</td>
</tr>
<tr>
<td>*Alkaline Potassium Iodide Azide Reagent</td>
<td>*7166-G</td>
</tr>
<tr>
<td>*Sulfuric Acid, 1:1</td>
<td>*6141WT-G</td>
</tr>
<tr>
<td>Sodium Thiosulfate, 0.025N</td>
<td>4169-H</td>
</tr>
<tr>
<td>Starch Indicator Solution</td>
<td>4170PS-G</td>
</tr>
<tr>
<td>Direct Reading Titrator, 0-10 Range</td>
<td>0377</td>
</tr>
<tr>
<td>Test Tube, 5-10-12.9-15-20-25 mL, glass, w/cap</td>
<td>0608</td>
</tr>
<tr>
<td>Pipet, plain, plastic, w/cap</td>
<td>0392</td>
</tr>
<tr>
<td>Water Sampling Bottle, 60 mL, glass</td>
<td>0688-DO</td>
</tr>
</tbody>
</table>

*WARNING: Reagents marked with an * are considered to be potential health hazards. See page 6 for more information.

The Titrator is calibrated in terms of Dissolved Oxygen expressed as ppm Dissolved Oxygen. Each minor division on the Titrator scale equals 0.2 ppm Dissolved Oxygen.
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.
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.
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 . . .
Insert the tip of the Titrator into the opening of the titration tube cap.

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.

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

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

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

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.

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.
**Hardness**

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Hardness Reagent #5</td>
<td>*4483-E</td>
</tr>
<tr>
<td>*Hardness Reagent #6 Solution</td>
<td>*4485-E</td>
</tr>
<tr>
<td>Hardness Reagent #7</td>
<td>4487DR-H</td>
</tr>
<tr>
<td>Test Tube, 5-10-12.9-15-20-25, glass, w/cap</td>
<td>0608</td>
</tr>
<tr>
<td>Direct Reading Titrator, 0-200 Range</td>
<td>0382</td>
</tr>
<tr>
<td>Pipet, 0.5 mL, plastic</td>
<td>0353</td>
</tr>
</tbody>
</table>

*WARNING: Reagents marked with an * are considered to be potential health hazards. See page 6 for more information.*

The Titrator is calibrated in terms of Total Hardness expressed as parts per million (ppm) Calcium Carbonate (CaCO₃). Each minor division on the Titrator scale equals 4 ppm CaCO₃.

**ANALYSIS OF HARDNESS IN SALT WATER**

When sea and estuarine waters containing very high levels of mineral salts are to be tested, the sample must be diluted before titration. This test set is supplied with a calibrated pipet for performing the simple, convenient dilution described below:

1. Use the 0.5 mL pipet (0353) to add 0.5 mL of the salt water to the test tube (0608).

2. Fill the test tube to the 12.9 mL line with distilled water (a 1 to 25.8 dilution).

1. Fill the test tube (0608) to the 12.9 mL line with the sample water.

2. Add 5 drops of *Hardness Reagent #5 (4483).

3. Cap and swirl to mix.

4. Add 5 drops of *Hardness Reagent #6 Solution (4485).

5. Cap and swirl to mix. Solution will turn red if hardness is present. If solution is blue, there is no measurable amount of hardness.

6. Fill Direct Reading Titrator (0382) with Hardness Reagent 7 (4487DR).

7. Insert the Titrator into the center hole of the test tube cap.

8. While gently swirling the tube, slowly press the plunger to titrate until red color changes to clear blue.

9. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as ppm Total Hardness (CaCO₃).
Nitrite Nitrogen

**DESCRIPTION**

<table>
<thead>
<tr>
<th></th>
<th>CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Mixed Acid Reagent</td>
<td>*V-6278-H</td>
</tr>
<tr>
<td>*Color Developing Reagent</td>
<td>*V-6281-D</td>
</tr>
<tr>
<td>Spoon, 0.1 g, plastic</td>
<td>0699</td>
</tr>
<tr>
<td>Test Tube, plastic, w/cap</td>
<td>0106</td>
</tr>
<tr>
<td>Dispenser Cap</td>
<td>0692</td>
</tr>
<tr>
<td>Octa-Slide 2 Viewer</td>
<td>1101</td>
</tr>
<tr>
<td>Nitrite Nitrogen Octa-Slide 2 Bar, 0.05-0.8 ppm</td>
<td>3437-01</td>
</tr>
</tbody>
</table>

*WARNING: Reagents marked with an * are considered to be potential health hazards. See page 6 for more information.

NOTE: Place Dispenser Cap (0692) on *Mixed Acid Reagent (V-6278-H). Save this cap for refill reagents.

1. Insert Nitrite-Nitrogen Octa-Slide 2 Bar (3437-01) into the Octa-Slide 2 Viewer (1101).

2. Fill a test tube (0106) to the 2.5 mL line with the water sample.

3. Dilute to the 5mL line with *Mixed Acid Reagent (V-6278).

4. Use the 0.1 g spoon (0699) to add 0.1 g of *Color Developing Reagent (V-6281).

5. Cap and mix by inverting for one minute.

6. Wait 5 minutes.

7. Insert test tube into Octa-Slide 2 Viewer (1101).

8. Match sample color to color standard. Record results as ppm Nitrite Nitrogen.

9. To convert to Nitrite, multiply results by 3.3. Record as ppm Nitrite. 

\[ \text{Nitrite-N (NO}_3^-\text{-N)} \times 3.3 = \text{ppm Nitrite (NO}_2^-) \]
**DESCRIPTION** | **CODE**
---|---
*Wide Range Indicator* | *2218-G*
Test Tube, plastic, w/cap | 0106
Octa-Slide 2 Viewer | 1101
Wide Range pH Octa-Slide 2 Bar, 5.0-10.0 | 3483-01

*WARNING: Reagents marked with an * are considered to be potential health hazards. See page 6 for more information.*

1. Insert Wide Range pH Octa-Slide 2 Bar (3483-01) into the Octa-Slide 2 Viewer (1101).

2. Fill a test tube (0106) to the 10 mL line with the water sample.

3. Add 8 drops of *Wide Range pH Indicator* (2218).

4. Cap and mix.

5. Insert test tube into Octa-Slide 2 Viewer (1101).

6. Match sample color to color standard. Record as pH.