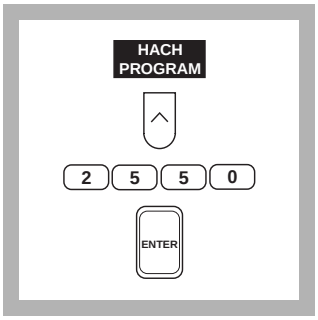
# NITROGEN, Total Inorganic

**Method 10021 Requires Centrifuge** **Titanium Trichloride Reduction Method**

**Test 'N Tube™ Vials** **(0 to 25.0 mg/L N)**

***Scope and Application:*** *For water, wastewater, and seawater.*
*The estimated detection limit for program number 2550 is 0.3 mg/L N.*




**1.** Press the soft key under ***HACH PROGRAM***.

Select the stored program number for total inorganic nitrogen, Test 'N Tube method, by pressing **2550** with the numeric keys.

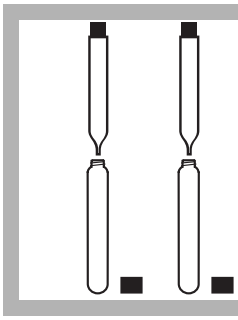
Press: **ENTER**

***Note:*** *If samples cannot be analyzed immediately, see Sample Collection, Preservation and storage following these steps. Adjust the pH of preserved samples before analysis.*

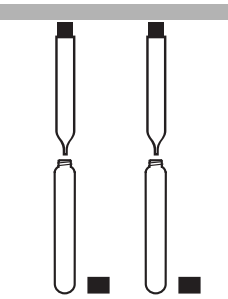

**2.** The display will show:

**HACH PROGRAM: 2550 N, Inorganic TNT**

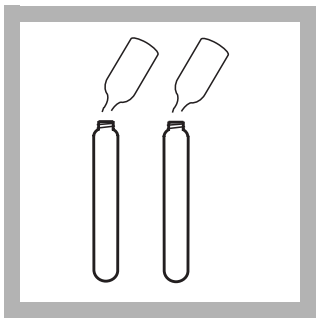
The wavelength (λ), **655 nm**, is automatically selected.



**3.** Pipet 1 mL of Total Inorganic Nitrogen Pretreatment Base Concentrate into each of 2 Total Inorganic Nitrogen Pretreatment Diluent Vials.



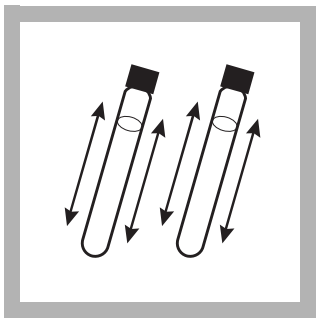
**4.** Pipet 1 mL of sample into 1 vial (the sample). Pipet 1 mL of deionized water into the other vial (the blank). Cap the vials and shake for 30 seconds to mix.



**5.** Snap the neck off two Total Inorganic Nitrogen Reductant ampules and pour the contents of one into the TIN Diluent Vial containing sample. Repeat for the second vial (the blank).

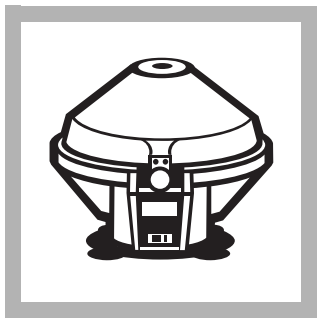
***Note:*** *For safety, wear gloves while breaking the ampules.*

***Note:*** *A black precipitate will form immediately.*



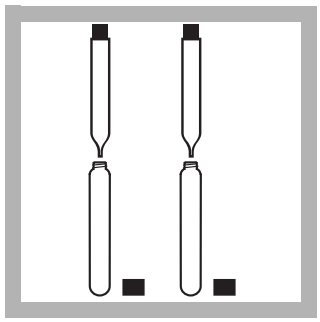
**6.** Cap the vials. Shake gently for 30 seconds to mix the reagents. Allow the vials to sit for at least one minute.

***Note:*** *The precipitate should remain black after shaking. Excessive shaking will result in a white precipitate and low results.*



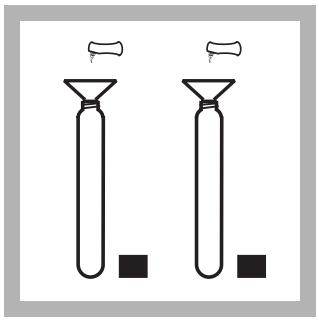
**7.** Centrifuge vials for 3 minutes or wait until solids settle to bottom of vial. Press the soft key under ***START TIMER*** to start a 3-minute period.

***Note:*** *The solids will settle without use of centrifuge, but can take up to 30 minutes.*

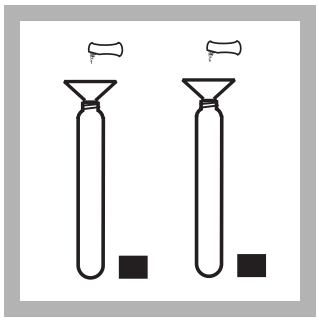


**8.** Remove the caps from two AmVer™ Diluent Reagent Test 'N Tubes for Low Range Ammonia Nitrogen. Using a pipet, add 2 mL of centrifuged sample to one vial. Add 2 mL of centrifuged blank to the other Test 'N Tube vial. Label the tubes appropriately.

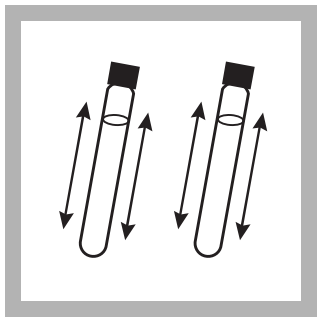
***Note:*** *Pipet carefully to avoid disturbing the sediment.*



**9.** Using a funnel add the contents of one Ammonia Salicylate Reagent Powder Pillow (for 5-mL sample) to each vial.

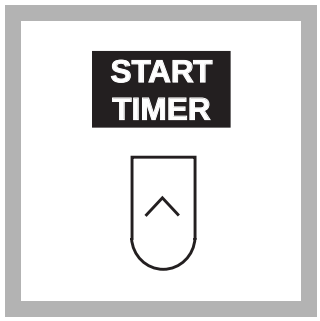


**10.** Using a funnel, add the contents of one Ammonia Cyanurate Reagent Powder Pillow (for 5-mL sample) to each vial.



**11.** Cap the vials tightly and shake thoroughly to dissolve the powder.

***Note:*** *A green color will develop if inorganic nitrogen is present.*



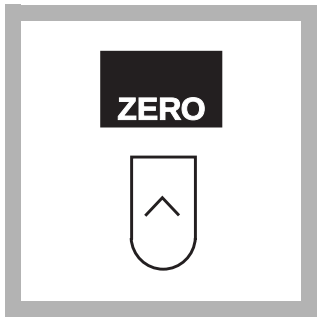

**12.** Press the soft key under ***START TIMER***.

A 20-minute reaction period will begin.

**13.** Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.

**14.** When the timer beeps, clean the outside of the vials with a towel, and place the blank into the cell holder.

***Note:*** *Wiping with a damp cloth followed with a dry removes fingerprints and other marks.*




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

The display will show:

**0.0 mg/L N**

***Note:*** *For alternate concentration units, press the soft key under* ***OPTIONS****. Then press the soft key under* ***UNITS*** *to scroll through the available options. Press* **ENTER** *to return to the read screen.*

**16.** Place the prepared sample into the cell holder and close the light shield. The result in mg/L total inorganic nitrogen as nitrogen (N) (or chosen units) will be displayed.

***Note:*** *The result can be expressed as N–$NH_3$ or $NO_3^-$. Press the soft keys under* ***OPTIONS,*** *then* ***FORM:*** *to scroll through the available options. Press* **ENTER** *to return to the read screen.*

## 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 |

## Sample Collection, Preservation 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 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. Neutralize with 5 N Sodium Hydroxide before analysis. Correct the test result for volume additions; see Section *1.2.2 Correcting for Volume Additions.*

## Accuracy Check

### Standard Additions Method

**a.** Leave the unspiked sample in the sample compartment. Verify that the units displayed are in mg/L. Select standard additions mode by pressing the soft keys under ***OPTIONS, (MORE)*** and then ***STD ADD***.

**b.** Press **ENTER** to accept the default sample volume (mL), 25.0.

**c.** Press **ENTER** to accept the default standard concentration (mg/L), 500.0.

**d.** Press the soft key under ***ENTRY DONE***.

**e.** Snap the neck off a fresh HR Nitrate Nitrogen PourRite Ampule Standard, 500-mg/L $NO_3^-$–N.

**f.** Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 25-mL samples and mix each thoroughly.

**g.** Analyze each standard addition sample as described above. Accept the standard additions reading by pressing the soft key under ***READ*** each time. Each addition should reflect approximately 90-95% recovery.

**h.** After completing the sequence, the display will show the extrapolated concentration value and the "best-fit" line through the standard additions data points, accounting for matrix interferences.

**i.** See Section *1.4.1 Standard Additions* for more information.

### Standard Solution Method

To check accuracy, use a 10.0-mg/L Nitrate Nitrogen Standard Solution listed under *OPTIONAL REAGENTS AND STANDARDS*. Or, prepare this by diluting 1 mL of solution from a Nitrate Nitrogen Voluette Ampule Standard, 500-mg/L $NO_3^-$–N, to 50 mL with deionized water. The result should be 9–10 mg/L N.

## Method Performance

**Precision/Accuracy**
The total inorganic nitrogen test is designed to provide an estimate of the total nitrite, nitrate, and ammonia nitrogen load present in a water or wastewater sample. This test is most applicable to the monitoring of samples taken from 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 does exhibit different recoveries of each of the three nitrogen species, as summarized below. The test is not recommended for use when quantifying only one of the three species. In that case, specific procedures for each particular analyte would be more appropriate.

**Ammonia Nitrogen**
In a single laboratory, using a standard solution of 20.0-mg/L $NH_3$-N and two representative lots of reagent with the instrument, a single operator obtained a mean recovery of 22.7 mg/L with a standard deviation of ± 0.88 mg/L N (replicate number = 7 per reagent lot).

**Nitrate Nitrogen**
In a single laboratory, using a standard solution of 20.0-mg/L $NO_3$–N and two representative lots of reagent with the instrument, a single operator obtained a mean recovery of 20.2 mg/L with a standard deviation of ± 0.67 mg/L N (replicate number = 7 per reagent lot).

**Nitrite Nitrogen**
In a single laboratory, using a standard solution of 20.0-mg/L $NO_2^-$–N and two representative lots of reagent with the instrument, a single operator obtained a mean recovery of 15.4 mg/L with a standard deviation of ± 0.74 mg/L N (replicate number = 7 per reagent lot).

**Estimated Detection Limit**

| Program | EDL |
|---|---|
| 2550 | 0.3 mg/L $NO_3^-$–N |

For more information on derivation and use of Hach's estimated detection limit, see Section *1.5.2*. To determine a method detection limit (MDL) as defined by the 40 *CFR* part 136, Appendix B, see Section *1.5.1*.

**Sensitivity**
Program Number: 2550

| Portion of Curve | ΔAbs | ΔConcentration |
|---|---|---|
| Entire Range | 0.010 | 0.16 mg/L |

See Section *1.5.3 Sensitivity Explained* for more information.

## Calibration Standard Preparation

To perform a total inorganic nitrogen calibration for the Test 'N Tube titanium trichloride reduction method, follow the steps under *Calibration Standard Preparation* in the LR Ammonia Test 'N Tube procedure. One change is required: You must enter the concentrations into the calibration table as 5, 10, 15, 20, and 25 mg/L TIN instead of the 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L $NH_3$–N stated. This will account for a 10-fold dilution in the total inorganic nitrogen method.

## 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.

## Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section *1*.

## Pollution Prevention and Waste Management

The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See Section *1* for further information in proper disposal of these materials.

## REQUIRED REAGENTS AND STANDARDS

| | Cat. No. |
|---|---|
| Total Inorganic Nitrogen Pretreatment Reagent Set ($TiCl_3$ Reduction Method) (25 tests) | 26049-45 |
| Includes: (1) 2040-59, (1) 26051-50, *(50) TIN Pretreatment Diluent Vials | |
| Test 'N Tube AmVer Nitrogen-Ammonia Reagent Set (25 tests) | 26045-45 |
| Includes: (1) 23952-66, (1) 23954-66, (1) 272-42, *(50) AmVer Diluent LR Vials | |

| Description | Quantity Required Per Test | Unit | Cat. No. |
|---|---|---|---|
| AmVer Diluent Reagent, Low Range Vials | 2 vials | 50/pkg | * |
| Ammonia Salicylate Reagent Powder Pillows | 2 pillows | 50/pkg | 23952-66 |
| Ammonia Cyanurate Reagent Powder Pillows | 2 pillows | 50/pkg | 23954-66 |
| Total Inorganic Nitrogen Pretreatment Diluent Vials | 2 vials | 50/pkg | * |
| Total Inorganic Nitrogen Reductant Ampule, 1 mL | 2 ampules | 50/pkg | 26051-50 |
| Total Inorganic Nitrogen Pretreatment Base Concentrate | 2 mL | 50 mL | 2040-59 |

* These items are not sold separately. Please order the complete set (Cat. No. 26049-45 or 26045-45) as a replacement.

## REQUIRED EQUIPMENT AND SUPPLIES

| | | | |
|---|---|---|---|
| Centrifuge, 115 VAC, 6 x 15 mL | 1 | each | 26765-00 |
| Centrifuge, 230 VAC, 6 x 15 mL | 1 | each | 26765-02 |
| DR/4000 Test Tube Adapter | 1 | each | 48189-00 |
| Funnel, micro | 1 | each | 25843-35 |
| Pipet, TenSette, 0 - 10 mL | 1 | each | 19700-10 |
| Pipet Tips, for 19700-10 Tensette Pipet | varies | 50/pkg | 21997-96 |
| Pipette, volumetric, Class A, 1.00-mL | 1 | each | 14515-35 |
| Test Tube Rack | 1 | each | 18641-00 |

## OPTIONAL REAGENTS AND STANDARDS

| | | | |
|---|---|---|---|
| Ammonia Nitrogen Standard Solution, 100-mg/L $NH_3$–N | 500 | mL | 24065-49 |
| 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, 2-mL Ampule, 500-mg/L $NO_3^-$–N | 20 | /pkg | 14260-20 |
| Sodium Hydroxide Standard Solution, 5.0 N | 59 | mL SCDB | 2450-26 |
| Sodium Thiosulfate Standard Solution, 0.1 N | 100 | mL MDB | 323-32 |
| Water, deionized | 4 | liters | 272-56 |

## OPTIONAL EQUIPMENT AND SUPPLIES

| | | | |
|---|---|---|---|
| Flask, volumetric, Class A, 50-mL | each | | 14574-41 |
| pH Indicator Paper, 1 to 11 pH | 5 | rolls/pkg | 391-33 |
| Pipet, volumetric, Class A, 5.00-mL | each | | 14515-37 |
| Pipet, volumetric, Class A, 10.00-mL | each | | 14515-38 |
| Pipet, volumetric, Class A, 15.00-mL | each | | 14515-39 |
| Pipet, volumetric, Class A, 20.00-mL | each | | 14515-20 |
| Pipet, volumetric, Class A, 25.00-mL | each | | 14515-40 |
| Pipet Filler | each | | 12189-00 |
| PourRite Ampule Breaker | each | | 24846-00 |

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