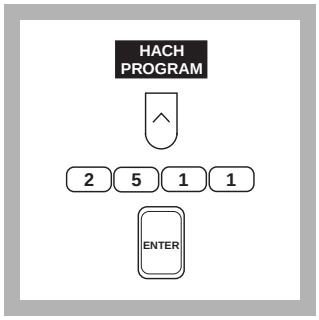
# NITRATE

**Method 10020** **Chromotropic Acid Method**

**Test 'N Tube™ Vials** **HR (0 to 30.0 mg/L $NO_3^-$–N)**

***Scope and Application:*** *For water and wastewater.*
*The estimated detection limit for program number 2511 is 0.2 mg/L $NO_3^-$–N.*




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

Select the stored program number for Nitrate, Test 'N Tube method, by pressing **2511** with the numeric keys.

Press: **ENTER**

***Note:*** *If samples cannot be analyzed immediately, see Sample Collection, Preservation and Storage following these steps.*

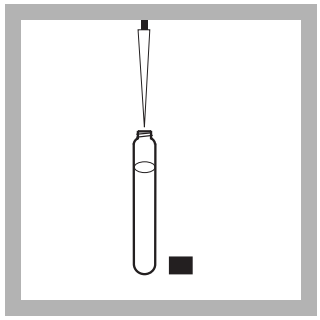
***Note:*** *The Flow Cell and Sipper Modules cannot be used with this method.*


**2.** The display will show:

**HACH PROGRAM: 2511 N, Nitrate HR TNT**

The wavelength (λ), **410 nm**, is automatically selected.

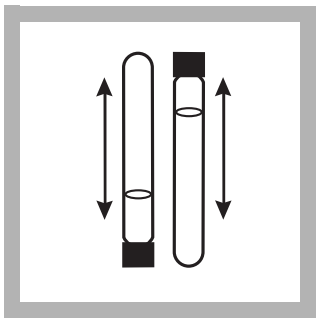
**3.** Insert the Test Tube Adapter into the sample cell module by sliding it under the thumb screw and into the alignment grooves. Fasten with the thumb screw.



**4.** Remove the cap from a Nitrate Pretreatment Solution Test 'N Tube vial and add 1.00 mL of sample (this will be the sample blank).

***Note:*** *For proof of accuracy, use a 15-mg/L Nitrate Nitrogen Standard Solution (listed under OPTIONAL REAGENTS AND STANDARDS) in place of the sample.*

***Note:*** *Run a reagent blank for this test. Use nitrate-free water in place of the sample. Subtract this result from all test results run with these lots of reagents. Determine a new reagent blank when the reagent lots change.*

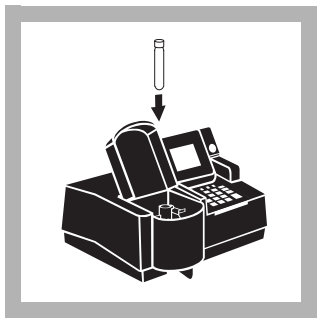


**5.** Cap the tube and invert it 10 times to mix.

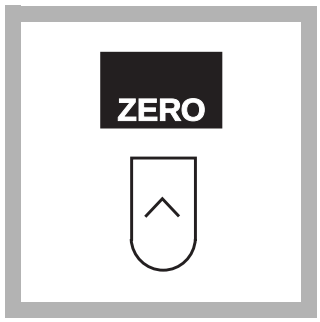
***Note:*** *This test is technique-sensitive. If these instructions are not followed, low results may occur. Hold the tube in a vertical position with the cap pointing up. Invert the vial so the cap now points down. Wait for all of the solution to flow to the cap end. Pause. Return the vial to the original position. Wait for all the solution to flow to the vial bottom. This process equals one inversion. Do this 10 times.*

**6.** Clean the outside of the vial with a towel.

***Note:*** *Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.*



**7.** Place the sample blank into the cell holder and close the light shield.

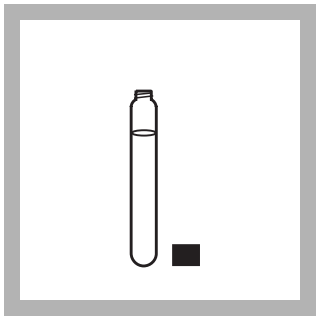



**8.** Press the soft key under ***ZERO***.

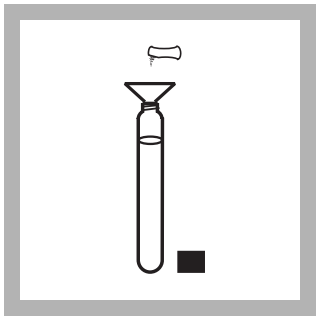
The display will show:

**0.0 mg/L $NO_3^-$–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.*



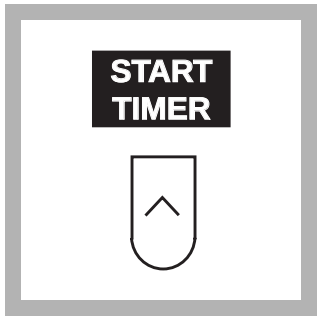
**9.** Remove the vial from the instrument. Remove the cap from the vial.



**10.** Using a funnel, add the contents of one NitraVer X Reagent B Powder Pillow to the vial. Cap and invert 10 times to mix. (This will be the prepared sample.)

***Note:*** *See Step 5 for inversion instructions.*

***Note:*** *Some solid matter will not dissolve.*




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

A 5-minute reaction period will begin. Do not invert the vial again.

***Note:*** *A yellow color will develop if nitrate nitrogen is present.*

***Note:*** *Complete steps 12-13 within 5 minutes after the timer beeps.*

**12.** When the timer beeps, clean the outside of the vial with a towel.

***Note:*** *Wiping with a damp towel, followed by a dry one, will remove fingerprints or other marks.*

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

***Note:*** *Measure the sample within 5 minutes after the timer beeps.*

***Note:*** *The results can be expressed as nitrate ($NO_3^-$) or $NO_3^-$–N. Press the soft keys under* ***METHOD OPTIONS****, then* ***FORM:*** *to scroll through the available options.*

## Interferences

**Table 1 Interfering Substances and Suggested Treatments**

| Interfering Substance | Interference Level |
|---|---|
| Barium | A negative interference at concentrations greater than 1 mg/L |
| Chloride | Does not interfere below 1000 mg/L |
| Hardness | Does not interfere |
| Nitrite | A positive interference at concentrations greater than 12 mg/L. Remove nitrite interference up to 100 mg/L by adding 400 mg of urea (one full 0.5 g Hach measuring spoon) to 10 mL of sample. Swirl to dissolve. Proceed with the nitrate test as usual. |

## Sample Collection, Preservation and Storage

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods (up to 14 days), adjust sample pH to 2 or less with concentrated sulfuric acid, ACS (about 2 mL per liter). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives.

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 High Range Nitrate Nitrogen Voluette Ampule Standard, 500-mg/L $NO_3^-$–N.

**f.** Use the TenSette Pipet to add 0.1, 0.2, 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 100% 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**
Use a 15.0-mg/L Nitrate Nitrogen Standard Solution listed under *OPTIONAL REAGENTS AND STANDARDS* to check test accuracy. Or, this can be prepared by pipetting 3.00 mL of solution from a High Range Nitrate Nitrogen 10-mL Voluette Ampule Standard Solution, 500-mg/L $NO_3^-$–N, into a 100-mL Class A volumetric flask. Dilute to the mark with deionized water.

## Method Performance

**Precision**
Standard: 15.0 mg/L $NO_3^-$–N

| Program | 95% Confidence Limits |
|---|---|
| 2511 | 14.8–15.2 mg/L $NO_3^-$–N |

For more information on determining precision data and method detection limits, refer to Section *1.5*.

**Estimated Detection Limit**

| Program | EDL |
|---|---|
| 2511 | 0.2 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: 2511

| Portion of Curve | ΔAbs | ΔConcentration |
|---|---|---|
| Entire Range | 0.010 | 0.22 mg/L |

See Section *1.5.3 Sensitivity Explained* for more information.

## Calibration Standard Preparation

To perform a nitrate calibration using the Test 'N Tube Chromotropic Acid method, prepare calibration standards containing 4, 14, and 30 mg/L $NO_3^-$–N as follows:

**a.** Into three different 500-mL Class A volumetric flasks, pipet 2.00, 7.00, and 15.00 mL of a 1000-mg/L Nitrate Nitrogen Standard Solution using Class A glassware.

**b.** Dilute to the mark with deionized water. Mix thoroughly.

**c.** Using the Test 'N Tube Chromotropic Acid method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

## Summary of Method

Nitrate in the sample reacts with chromotropic acid under strongly acidic conditions to yield a yellow product with a maximum absorbance at 410 nm.

## 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

For information on pollution prevention and waste management, refer to Section *1*.

## REQUIRED REAGENTS AND STANDARDS

| | Cat. No. |
|---|---|
| Test 'N Tube NitraVer X Nitrate Reagent Set (50 tests) | 26053-45 |
| Includes: (1) 26055-46, (1) 272-42, (50) Nitrate Pretreatment Solution Vials* | |

| Description | Quantity Required Per Test | Unit | Cat. No. |
|---|---|---|---|
| Nitrate Pretreatment Solution Vials | 1 | 50/pkg | * |
| NitraVer X Reagent B Powder Pillows | 1 | 50/pkg | 26055-46 |

## REQUIRED EQUIPMENT AND SUPPLIES

| | | | |
|---|---|---|---|
| DR/4000 Test Tube Adapter | 1 | each | 48189-00 |
| Funnel, micro, poly | 1 | each | 25843-35 |
| Pipet, TenSette, 0.1 to 1.0 mL | 1 | each | 19700-01 |
| Pipet Tips, for 19700-01 TenSette Pipet | varies | 50/pkg | 21856-96 |
| Test Tube Rack, cooling | 1-3 | each | 18641-00 |

## OPTIONAL REAGENTS AND STANDARDS

| | | |
|---|---|---|
| Nitrate Nitrogen Standard Solution, 15-mg/L N | 100 mL MDB | 24151-32 |
| Nitrate Nitrogen Standard Solution, 1000-mg/L N | 500 mL | 12792-49 |
| Nitrate Nitrogen Standard Solution, Voluette Ampule, 500-mg/L N | 16/pkg | 14260-10 |
| Sodium Hydroxide Standard Solution, 5.0 N | 50 mL | 2450-26 |
| Sulfuric Acid, concentrated, ACS | 500 mL | 979-49 |
| Urea, ACS | 100 g | 11237-26 |
| Water, deionized | 4 liters | 272-56 |

## OPTIONAL EQUIPMENT AND SUPPLIES

| | | |
|---|---|---|
| Ampule Breaker Kit | each | 21968-00 |
| Flask, volumetric, Class A, 100-mL | each | 14574-42 |
| Flask, volumetric, Class A, 500-mL | each | 14574-49 |
| pH Paper, pH 1.0 to 11.0 | 5 rolls/pkg | 391-33 |
| Pipet, serological, 2-mL | each | 532-36 |
| Pipet, volumetric, Class A, 2.00-mL | each | 14515-36 |
| Pipet, volumetric, Class A, 3.00-mL | each | 14515-03 |
| Pipet, volumetric, Class A, 7.00-mL | each | 14515-07 |
| Pipet, volumetric, Class A, 15.00-mL | each | 14515-39 |
| Pipet Filler | each | 12189-00 |
| Spoon, measuring, 0.5-g | each | 907-00 |

* Items not sold separately.

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