

Chromotropic Acid Method
0.2 to 30.0 mg/L NO₃⁻-N (HR)

Method 10020
Test 'N Tube™ Vials

Scope and application: For 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 adapter and light shield requirements for the instruments that use them.

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

Table 1 Instrument-specific information for test tubes

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800, DR 2700	—	LZV646
DR 1900	9609900 (D ¹)	—
DR 900	4846400	Cover supplied with the instrument

Before starting

Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.

DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before this test is started.

The vials must be mixed carefully for accurate results. Start each vial inversion with the vial in the vertical position, with the cap on the top. Turn the vial upside-down and wait for all of the solution to flow down to the cap. Return the vial to the vertical position and wait for all of the solution to flow down to the bottom of the vial. This mixing method equals one inversion.

For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option.

UV light changes the color of the prepared sample to yellow. Keep the prepared sample out of direct sunlight.

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
Light shield (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
NitraVer ^{®2} X Nitrate Reagent Set, Test 'N Tube™	1

¹ The D adapter is not available with all instrument versions.

² NitraVer is a registered trademark of Hach Company

Items to collect (continued)

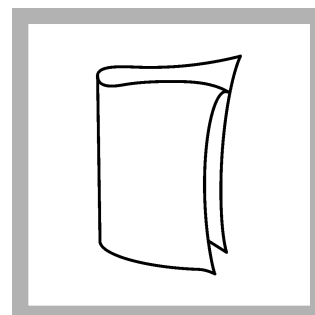
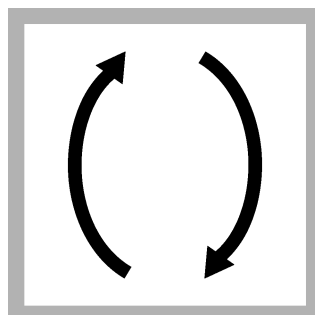
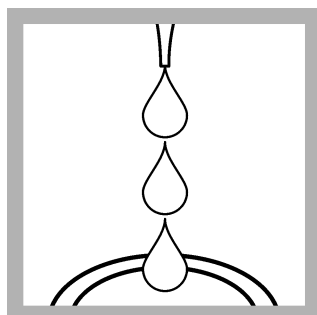
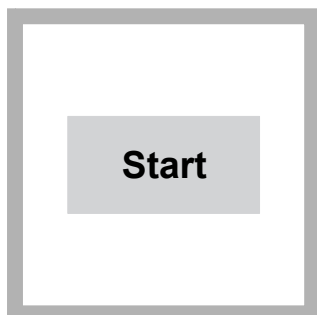
Description	Quantity
Funnel, micro, poly	1
Pipet, TenSette [®] , 0.1–1.0 mL	1
Test tube rack	1

Refer to [Consumables and replacement items](#) on page 4 for order information.

Sample collection and storage

- Collect samples in clean glass or plastic bottles.
- Analyze the samples as soon as possible for best results.
- If immediate analysis is not possible, immediately filter and keep the samples at or below 6 °C (43 °F) for a maximum of 48 hours.
- To preserve samples for a maximum of 28 days, adjust the sample pH to 2 or less with concentrated sulfuric acid (approximately 2 mL per liter) and keep at or below 6 °C (43 °F). The test results then include nitrate and nitrite.
- Let the sample temperature increase to room temperature before analysis.
- Before analysis, adjust the pH to 7 with 5 N sodium hydroxide solution.
- Correct the test result for the dilution caused by the volume additions.

TNT procedure



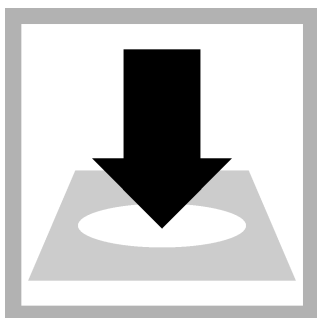
1. Start program **344 N, Nitrate HR, TNT**. For information about sample cells, adapters or light shields, refer to [Instrument-specific information](#) on page 1.

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

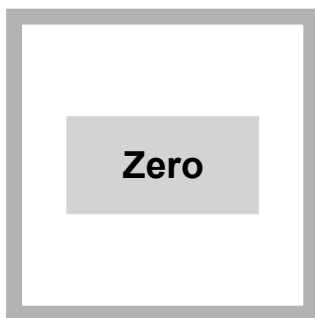
2. **Prepare the blank:** Use a pipet to add 1.00 mL of sample to a NitraVer X Reagent A Test 'N Tube vial.

3. Close the vial. Invert the vial 10 times to mix.

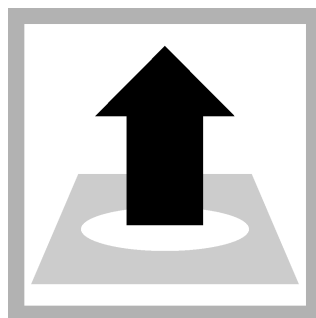
4. Clean the blank vial.



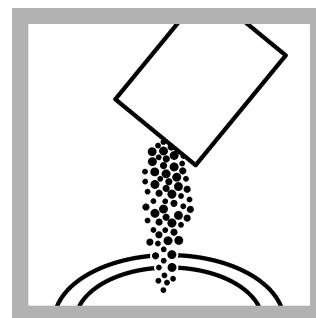
5. Insert the vial into the cell holder.



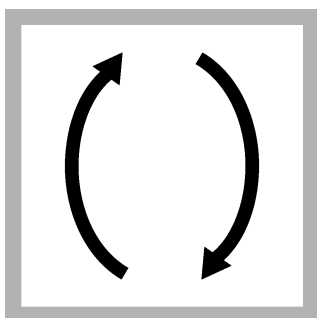
6. Push **ZERO**. The display shows 0.0 mg/L NO_3^- -N.



7. Remove the TNT vial from the cell holder.



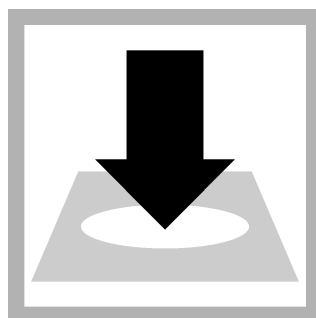
8. **Prepare the sample:** Use a funnel to add the contents of one NitraVer X Reagent B Powder Pillow to the vial.



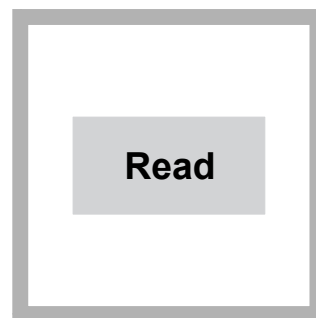
9. Close the vial. Invert the vial 10 times to mix. Some solid matter will not dissolve.



10. Start the instrument timer. A 5-minute reaction time starts. Do not invert the vial again. A yellow color shows if nitrate is present.



11. Within 5 minutes after the timer expires, clean the vial. Insert the vial into the cell holder.



12. Push **READ**. Results show in mg/L NO_3^- -N.

Interferences

Interfering substance	Interference level
Barium	Negative interference at more than 1 mg/L
Chloride	Does not interfere at less than 1000 mg/L
Copper	Positive interference at all levels
Nitrite	A positive interference at concentrations more than 12 mg/L. To remove nitrite interference up to 100 mg/L, add 400 mg (one full 0.5 g measuring spoon) of urea to 10 mL of sample. Swirl to dissolve. Proceed with the nitrate test as usual.
Interference from direct sunlight	UV light changes the color of the prepared sample to yellow. Keep the prepared sample out of direct sunlight.

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:

- 500 mg/L Nitrate Nitrogen Standard Solution, Voluette® Ampule
- Ampule breaker
- Pipet, TenSette®, 0.1–1.0 mL and tips
- 25-mL mixing cylinders (3)

1. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
2. Go to the Standard Additions option in the instrument menu.
3. Select the values for standard concentration, sample volume and spike volumes.
4. Open the standard solution.
5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard solution, respectively, to three 25-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:

- 10.0 mg/L Nitrate Nitrogen Standard Solution

1. Use the test procedure to measure the concentration of the standard solution.
2. 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
344	10.0 mg/L NO ₃ ⁻ -N	9.5–10.5 mg/L NO ₃ ⁻ -N	0.2 mg/L NO ₃ ⁻ -N

Summary of method

Nitrate in the sample reacts with chromotropic acid under strongly acidic conditions to yield a yellow product. The measurement wavelength is 410 nm for spectrophotometers or 420 nm for colorimeters.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
NitraVer ^{®3} X Nitrate Reagent Set, Test 'N Tube™	—	50 tests	2605345

³ NitraVer is a registered trademark of Hach Company

Required apparatus

Description	Quantity/test	Unit	Item no.
Funnel, micro, poly	1	each	2584335
Pipet, TenSette [®] , 0.1–1.0 mL	1	each	1970001
Pipet tips, for TenSette [®] Pipet, 0.1–1.0 mL	2	50/pkg	2185696
Test tube rack	1	each	1864100

Recommended standards

Description	Unit	Item no.
Nitrate Nitrogen Standard Solution, 10.0-mg/L NO ₃ -N	500 mL	30749
Nitrate Nitrogen Standard Solution, Voluette [®] Ampule, 500-mg/L NO ₃ -N	16/pkg	1426010
Wastewater Influent Standard Solution, Mixed Parameter, for NH ₃ -N, NO ₃ -N, PO ₄ , COD, SO ₄ , TOC	500 mL	2833149
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Item no.
Ampule Breaker, 10-mL Voluette [®] Ampules	each	2196800
Mixing cylinder, graduated, 25-mL	each	2088640
Pipet tips for TenSette [®] Pipet, 0.1–1.0 mL	1000/pkg	2185628
Sodium Hydroxide Solution, 5 N	50 mL	245026
Spoon, measuring, 0.5-g	each	90700
Sulfuric Acid, ACS	500 mL	97949
Urea, ACS grade	100 g	1123726



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