Nitrogen, Total

DOC316.53.01085

Persulfate Digestion Method **Method 10072**

2 to 150 mg/L N (HR) **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.
Digestion is required for total nitrogen determinations.
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.
If the test result is over-range, dilute a fresh portion of sample and repeat the complete test procedure. The digestion must be repeated for accurate results.
Use the deionized water that is supplied in the reagent set or organic-free water for the blank vial and for the preparation of standard solutions.
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.

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

Items to collect

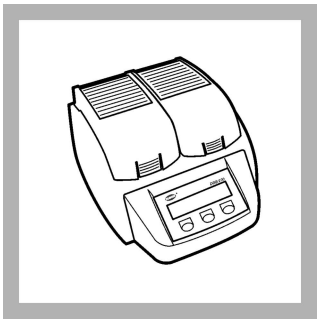
Description	Quantity
Test 'N Tube HR Total Nitrogen Reagent Set	1
DRB200 Reactor	1
Finger cots	2
Funnel, micro	1
Light shield or adapter (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Pipet, TenSette®, 0.1- to 1.0-mL, with pipet tips	1
Pipet, TenSette®, 1.0- to 10.0-mL, with pipet tips	1
Test tube rack	1

Refer to Consumables and replacement items on page 7 for order information.

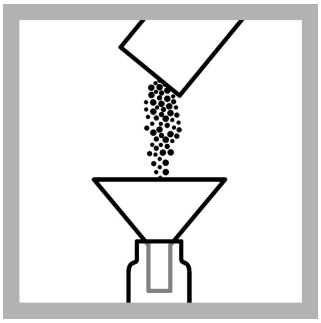
Sample collection and storage

- Collect samples in clean glass or plastic bottles.
- Analyze the samples as soon as possible for best results.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated sulfuric acid (about 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at or below 6 °C (43 °F) for a maximum of 28 days.
- Let the sample temperature increase to room temperature before analysis.
- Before analysis, adjust the pH to 7 with 5.0 N sodium hydroxide standard solution.
- Correct the test result for the dilution caused by the volume additions.

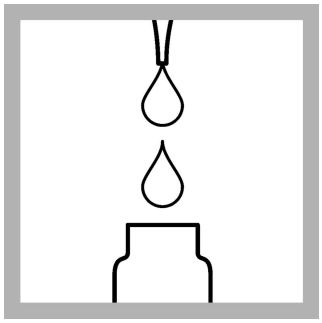
Persulfate digestion for Test 'N Tubes



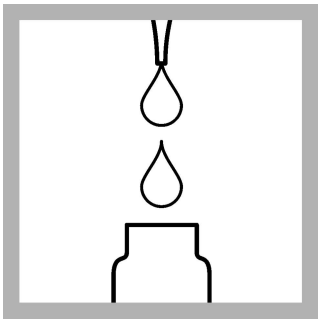
1. Start the DRB200 Reactor. Set the temperature to 105 °C.



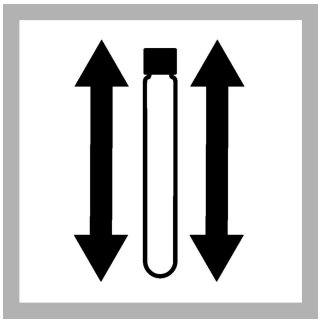
2. Use a funnel to add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two HR Total Nitrogen Hydroxide Digestion Reagent vials.
Make sure to clean any reagent that gets on the lip of the vials or on the vial threads.



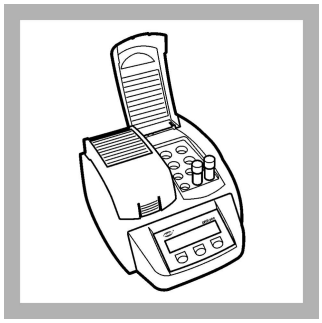
3. Prepare the sample: Add 0.5 mL of sample to one of the vials.



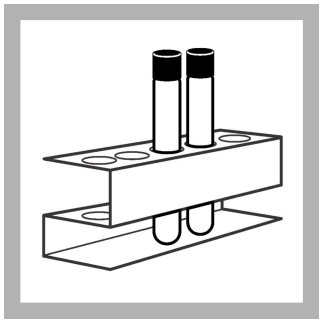
4. Prepare the blank: Add 0.5 mL of deionized water (included in the kit) to the second vial.
Use only water that is free of all nitrogen-containing species as a substitute for the provided deionized water.



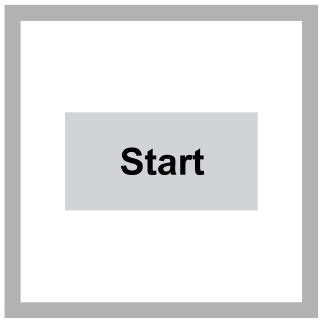
5. Put the caps on both vials. Shake vigorously for at least 30 seconds to mix. Undissolved powder will not affect the accuracy of the test.



6. Put the vials in the reactor and close the lid. Leave the vials in the reactor for exactly 30 minutes.

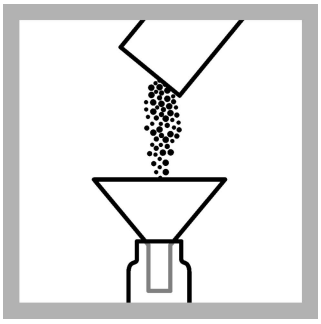


7. At 30 minutes, use finger cots to immediately remove the vials from the reactor. Let the vials cool to room temperature.

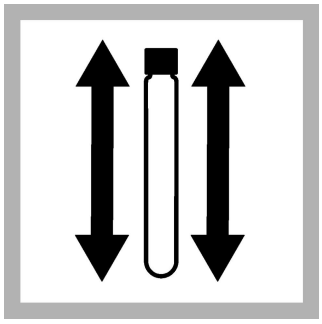


8. Start program **394 N, Total HR TNT**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.

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

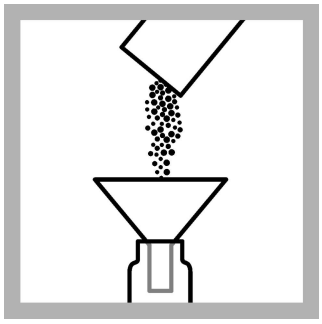


9. Add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.

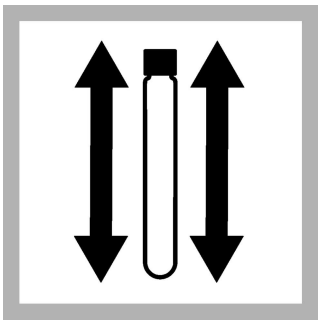


10. Put the caps on both vials. Shake for 30 seconds.

11. Start the instrument timer. A 3-minute reaction time starts.

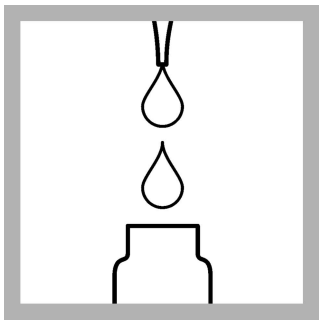


12. After the timer expires, remove the caps from the vials. Add one TN Reagent B Powder Pillow to each vial.

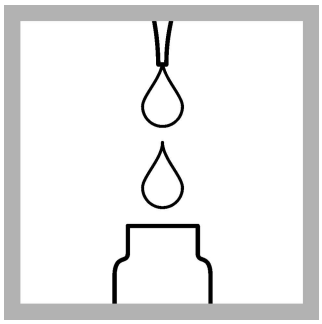


13. Put the caps on both vials. Shake vigorously for 15 seconds to mix. The reagent will not dissolve completely. Undissolved powder will not affect the accuracy of the test.
The solution will start to turn yellow.

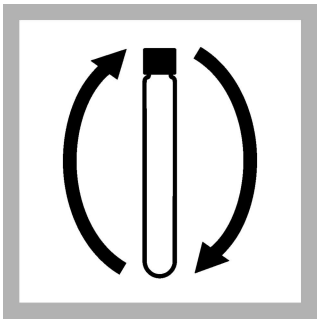
14. Start the instrument timer. A 2-minute reaction time starts.



15. Prepared sample: When the timer expires, use a pipet to put 2 mL of the digested, treated prepared **sample** into one TN Reagent C vial.

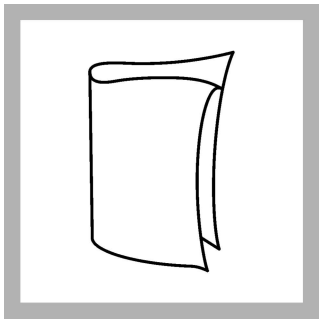


16. Blank: When the timer expires, use a pipet to put 2 mL of the digested, treated **blank** into the second TN Reagent C vial.

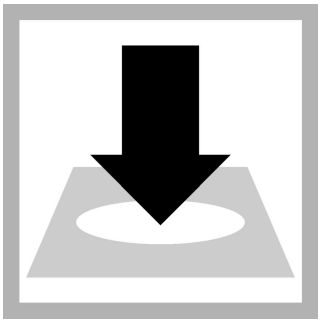


17. Put the caps on both vials. Invert 10 times to mix. Use slow, deliberation inversions for complete recovery. The vials will be warm to the touch.

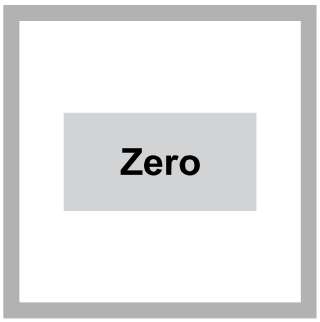
18. Start the instrument timer. A 5-minute. reaction time starts.
The yellow color will intensify.



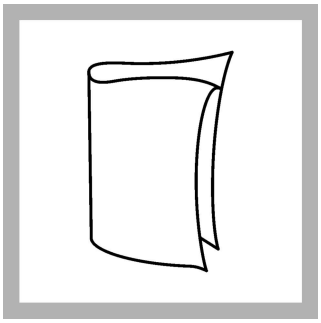
19. When the timer expires, clean the blank vial.



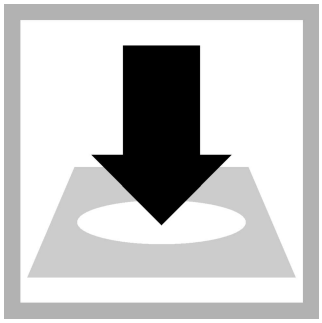
20. Insert the blank vial into the 16-mm cell holder.



21. Push **ZERO**. The display shows 0 mg/L N.



22. Clean the sample vial.



23. Insert the sample vial into the 16-mm cell holder.

24. Push **READ**. Results show in mg/L N.

Blanks for colorimetric measurement

The reagent blank can be used for up to 7 days for measurements that use the same lot of reagents. Keep the reagent blank in the dark at room temperature (18–25 °C). If a small amount of white floc appears within a week, discard the reagent blank and prepare a new one.

Interferences

The substances in the Table 2 have been tested and found not to interfere up to the indicated levels (in mg/L). Interfering substances that resulted in a concentration change of ±10% appear in the Table 3.

Table 2 Non-interfering substances

Interfering substance	Interference level
Barium	10.4 mg/L
Calcium	1200 mg/L
Chromium ($^{3+}$)	2 mg/L
Iron	8 mg/L
Lead	26.4 µg/L
Magnesium	2000 mg/L
Organic Carbon	600 mg/L
Phosphorus	400 mg/L
Silica	600 mg/L

Table 2 Non-interfering substances (continued)

Interfering substance	Interference level
Silver	3.6 mg/L
Tin	6 mg/L

Table 3 Interfering substances

Interfering substance	Interference level
Bromide	> 240 mg/L; positive interference
Chloride	> 3000 mg/L; positive interference
Interference from direct sunlight	UV light changes the color of the prepared sample to yellow. Keep the prepared sample out of direct sunlight.

This test performed with standard nitrogen solutions prepared from the following compounds obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Glycine
- Urea

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in ≥ 95% recovery.

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate reagent. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Accuracy check

Digestion method

For proof of accuracy use Primary Standards for Kjeldahl Nitrogen. This method generally gives 95–100% recovery on organic nitrogen standards. Analysts have found Nicotinic acid-PTSA (p-Toluenesulfonate) to be the most difficult to digest. Other compounds may yield different percent recoveries.
Items to collect:

- Primary Standard for Kjeldahl Nitrogen (Ammonia-PTSA, Glycine-PTSA or Nicotinic-PTSA)
- 1-L volumetric flask, Class A
- Deionized water (use the deionized water supplied in the reagent set or water that is free of all organic and nitrogen-containing species)

1. Prepare a 120-mg/L N equivalent standard.
   a. Weigh the applicable standard:
      - Ammonia-PTSA: 1.6208 g
      - Glycine-PTSA: 2.1179 g
      - Nicotinic-PTSA: 2.5295 g
   b. Use a funnel to add the standard to the volumetric flask.
   c. Add deionized water to the flask and mix to dissolve the standard.
   d. Dilute to the mark with deionized water. Mix well.
2. Use the test procedure to measure the concentration of the nitrogen standard. Calculate the percent recovery as follows:

% recovery = [(measured concentration)/120] x 100

Note: *The minimum expected % recovery for each standard is 95%.*

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:

- Ammonia Nitrogen Standard Solution, 1000-mg/L as NH_3–N
- 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:

- 100-mg/L ammonia 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
394	100 mg/L NH_3–N	98–102 mg/L N	0.5 mg/L N

Summary of method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow

complex. 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.
Nitrogen, Total, Test 'N Tube™ Reagent Set		50 vials	2714100

Required apparatus

Description	Quantity/test	Unit	Item no.
DRB 200 Reactor, 110 VAC option, 15 x 16-mm wells	1	each	LTV082.53.40001
OR			
DRB 200 Reactor, 220 VAC option, 15 x 16-mm wells	1	each	LTV082.52.40001
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
Pipet, TenSette® 1.0–10.0 mL	1	each	1970010
Pipet tips, for TenSette® Pipet, 1.0–10.0 mL	varies	50/pkg	2199796
Test tube rack	1	each	1864100
Finger cots	2	2/pkg	1464702

Recommended standards and apparatus

Description	Unit	Item no.
Nitrogen Ammonia Standard Solution, 1000-mg/L as NH_3–N	1 L	2354153
Nitrogen Ammonia Standard Solution, 100-mg/L as NH_3–N	500 mL	2406549
Balance, analytical, 80 g x 0.1 mg 100–240 VAC	each	2936701
Mixing cylinder, graduated, 25-mL	each	2088640
Flask, volumetric, Class A, 1000-mL glass	each	1457453
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Pipet tips for TenSette® Pipet, 1.0–10.0 mL	250/pkg	2199725
Kjeldahl Nitrogen Primary Standard Set	set of 3	2277800
Sodium Hydroxide Solution, 5 N	50 mL	245026
Sulfuric Acid, ACS	500 mL	97949
Wastewater Influent Standard Solution, Mixed Parameter, for NH_3-N, NO_3-N, PO_4, COD, SO_4, TOC	500 mL	2833149
Water, deionized	500 mL	27249
Water, organic-free	500 mL	2641549
Paper, for weighing, 100 x 100 mm	500/pkg	1473885
PourRite® Ampule Breaker, 2-mL	each	2484600
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800
Nitrogen Ammonia Standard Solution, 10-mL Voluette® Ampule, 50-mg/L NH_3–N	16/pkg	1479110
Nitrogen Ammonia Standard Solution, 150-mg/L NH_3–N, 10-mL Voulette® Ampules	16/pkg	2128410

Recommended standards and apparatus (continued)

Description	Unit	Item no.
Nitrogen Ammonia Standard Solution, 2-mL PourRite® Ampules, 50-mg/L	20/pkg	1479120
Nitrogen, Ammonia Standard Solution, 10-mg/L NH_3-N	500 mL	15349

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