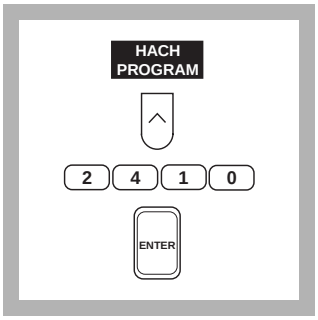
NITROGEN, Total Kjeldahl

Method 8075 **Nessler Method* (Digestion Required)**

(0 to 150.0 mg/L)

Scope and Application: *For water, wastewater and sludge; digestion is required for determining total kjeldahl nitrogen. The estimated detection limit for program number 2410 is 1.2 mg/L TKN.*

* Adapted from Hach, et. al., *Journal of Association of Official Analytical Chemists, 70 (5) 783-787* (1987); Hach, et. al., *Journal of Agricultural and Food Chemistry, 33* (6) 1117-1123 (1985); *Standard Methods for the Examination of Water and Wastewater*



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

Select the stored program for total kjeldahl nitrogen by pressing **2410** with the numeric keys.

Press: **ENTER**

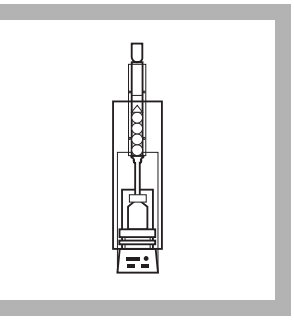
Note: *The Flow Cell and Sipper Modules can be used with this procedure. If the Flow-Thru Cell is used, periodically clean the cell by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel. Flush it through the funnel and cell with enough deionized water to dissolve. Rinse out the crystals.*

2. The display will show:

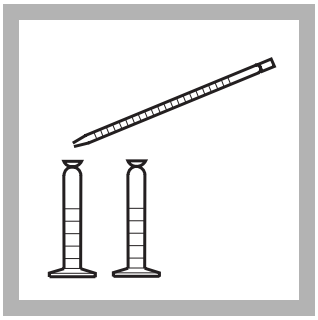
HACH PROGRAM: 2410
Nitrogen, TKN

The wavelength (λ), **460 nm**, is automatically selected.

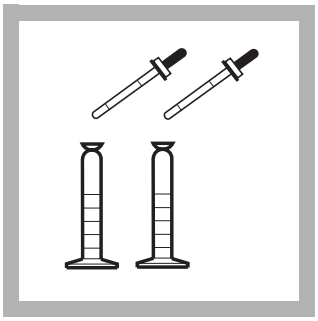
Note: *For best results, determine a reagent blank for each new lot of reagent as follows. Prepare a reagent blank by repeating steps 3 through 15, using deionized water as the sample. Zero the instrument on deionized water by pressing the soft key under* ***ZERO****. Insert the reagent blank and the blank value will be displayed. Correct for the reagent blank by pressing the soft keys under* ***OPTIONS, (MORE)****, and then* ***BLANK:OFF****. Enter the reagent blank value and press* **ENTER***. Repeat for each new lot of reagent.*



3. Digest the sample amount as described in the *Digesdahl Digestion Apparatus Instruction Manual*. Digest an equal amount of deionized water as the blank.



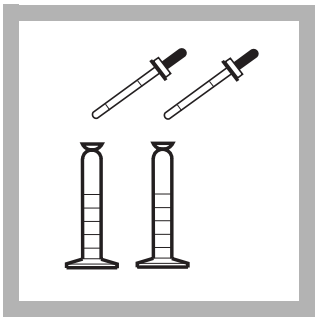
4. Select the appropriate analysis volume of the digested sample given in *Table 1* on page *3*. Pipet the analysis volume from the sample and the blank into separate 25-mL mixing graduated cylinders.



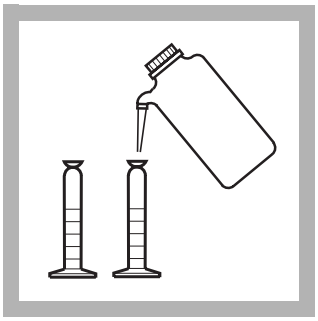
5. Add one drop of TKN Indicator to each cylinder. Add drops of 8.0 N KOH to each cylinder until the first flash of blue color appears. Stopper and invert the cylinder each addition.

Note: *If aliquot is less than 1 mL, do not add KOH. Continue with Step 6.*

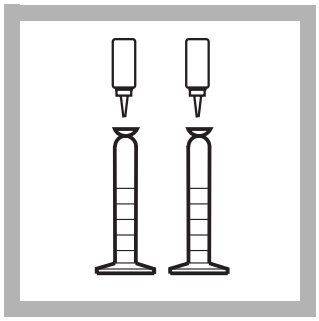
Note: *Hold the dropping bottles upright while dispensing.*



6. Add 1.0 N KOH to each cylinder, one drop at a time, mixing after each addition. Continue until the first permanent blue color appears.

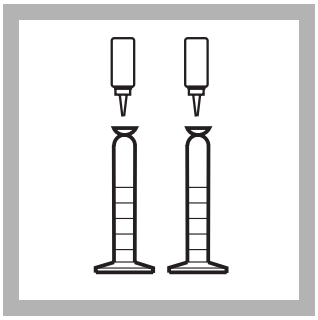


7. Fill both cylinders to the 20-mL mark with deionized water.



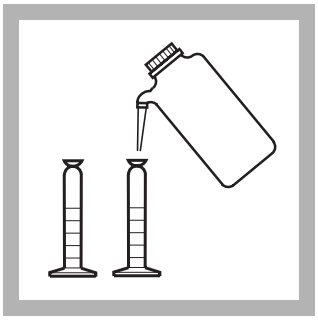
8. Add three drops of Mineral Stabilizer to each cylinder. Stopper. Invert several times to mix.

Note: *Hold the dropping bottles upright while dispensing.*

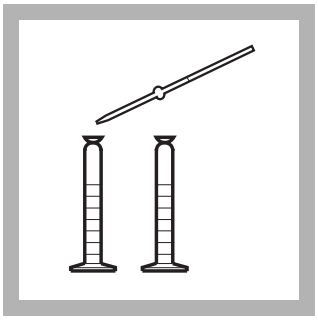


9. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder. Stopper. Invert several times to mix.

Note: *Hold the dropping bottles upright while dispensing.*

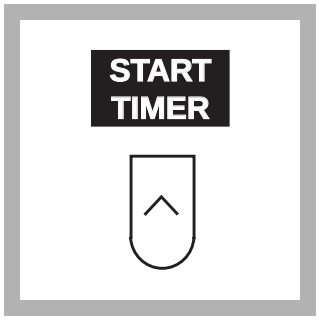


10. Fill both cylinders to the 25-mL mark with deionized water. Stopper. Invert several times to mix.



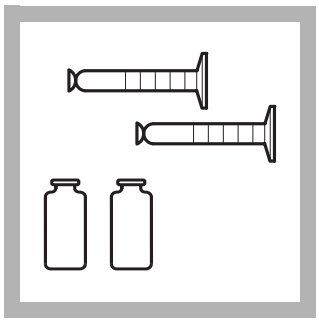
11. Pipet 1.00 mL of Nesslers Reagent to each cylinder. Stopper, invert repeatedly. The solution should not be hazy.

Any haze (turbidity) will cause inaccurate results.

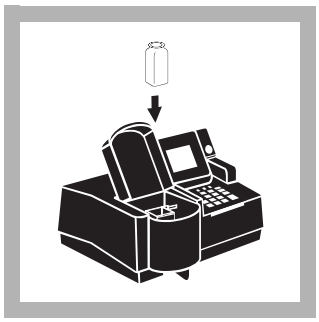


12. Press the soft key under ***START TIMER***.

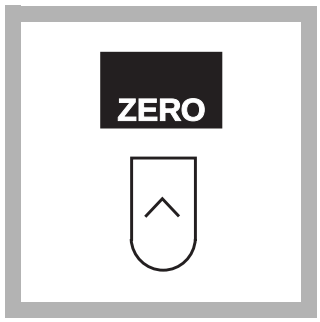
A 2-minute reaction period will begin.



13. When the timer beeps, pour the contents of each cylinder into separate 25-mL sample cells.



14. Place the blank into a cell holder. Close the light shield.



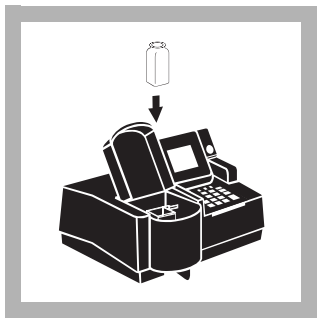
15. Press the soft key under ***ZERO***.

The display will show:

0.0 mg/L TKN

Note: *If you are using a reagent blank correction, the display will show the correction.*

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. Close the light shield. Results in mg/L total kjeldahl nitrogen as N (or chosen units) will be displayed.

Note: *The readout is the actual concentration of total kjeldahl nitrogen when the sample amount is 25 mL and the analysis volume is 3 mL. If other volumes are used, the true concentration must be calculated using the formula in Step 17*

Note: *The results can be expressed as NH_3, NH_4^+ or NH_3–N. Press the soft keys under* ***OPTIONS****, then* ***FORM:*** *to scroll through the available options. Press* **ENTER** *to return to the read screen.*

Note: *See Pollution Prevention and Waste Management following these steps about disposal of these reagents.*

17. Calculate sample TKN as follows:

$$\text{ppm TKN} = \frac{75 \times A}{B \times C}$$

Where:

A = mg/L read from the display
B = g (or mL of water) sample taken for digest
C = mL analysis volume of digested sample.

Table 1 Digestion Table

AQUEOUS SAMPLES (Solutions of suspensions in water—less than 1% solids)	
Expected Nitrogen Concentration (mg/L)	Analysis Volume (mL)
0.5–28	10.0
2–112	5.0
11–560	2.00
45–2250	1.00
425–22500	0.500
DRY SAMPLES	
Expected Nitrogen Concentration (mg/L)	Analysis Volume (mL)
42–2200	10.0
106–5600	5.00
350–18000	2.00
1000–56000	1.00
4200–220000	0.50
OILS AND FATS	
Expected Nitrogen Concentration (mg/L)	Analysis Volume (mL)
85–4500	10.0
210–11000	5.00
2100–110000	1.00

Sample Collection, Storage and Preservation

Collect samples in clean glass or plastic containers. Adjust the pH to 2 or less with sulfuric acid (about 2 mL per liter) and cool to 4 °C. Preserved samples can be stored up to 28 days.

Accuracy Check

Kjeldahl Nitrogen Standard Method
This procedure checks digestion efficiency and indicates that amount of bound nitrogen that is freed during digestion. The methods and standards available to check digestion technique are found in the *Accuracy Check* section following the procedure in the *Digesdahl digestion Apparatus Instruction Manua*l. Using the digested Kjeldahl standard, perform the above TKN analysis on the colorimeter. The TKN value should come within about ±3% of the value of the prepared Kjeldahl standard.

Standard Solution Method (to check calibration accuracy only)
Add one drop of TKN Indicator to each of two 25-mL graduated mixing cylinders. Fill one cylinder to the 20-mL mark with deionized water. Fill the other cylinder to the 20-mL mark with a 1.0 mg/L NH_3-N solution. Add 3 drops of Mineral Stabilizer to each cylinder. Invert several times to mix. Add 3 drops of Polyvinyl Alcohol Dispersing agent to each cylinder. Perform the TKN procedure as described in steps 10 to 16. This display should show 26–27 mg/L TKN.

Method Performance

Precision
Standard: 35.0 mg/L NH_3–N

Program	95% Confidence Limits
2410	34.3–35.7 mg/L NH_3–N

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

Estimated Detection Limit

Program	EDL
2410	1.2 mg/L NH_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: 2410

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	1.02 mg/L

See Section *1.5.3 Sensitivity Explained* for more information.

Calibration Standard Preparation

A new calibration may be performed for each lot of Nessler Reagent.

Prepare standards representing 28, 56, 84, 112 and 140 mg/L N as follows:

a. Into five different 100-mL Class A volumetric flasks, pipet 7, 14, 21, 28, and 35 mL of a 100-mg/L Ammonia Nitrogen Standard Solution (Cat. No. 24065-49) using Class A glassware.

b. Dilute to the mark with deionized water. Mix thoroughly. These standards are prepared as though the digestion were performed.

c. Beginning at Step 4, use a 3-mL analysis volume and complete the procedure.

d. This calibration can be stored as a ***USER PROGRAM***. For more information, refer to the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*.

Summary of Method

The term “Total Kjeldahl Nitrogen” refers to the combination of ammonia and organic nitrogen. However, only the organic nitrogen compounds appearing as organically bound nitrogen in the trinegative state are determined in this test. Nitrogen in this form is converted into ammonium salts by the action of sulfuric acid and hydrogen peroxide. The ammonia is then analyzed by a modified Nessler method test.

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

Nessler reagent contains mercuric iodide. Both the sample and blank will contain mercury (D009) at concentrations regulated as a hazardous waste by the Federal RCRA. Do not pour these solutions down the drain. See Section *1* for more information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

Kjeldahl Nitrogen Reagent Set.....24953-00
Includes: (1) 21196-49, (1) 23766-26, (1) 21194-49, (1) 23765-26, (1) 23144-26, (1) 282-32, (1) 979-49, (1) 22519-26

Description	Quantity Required Per Test	Unit	Cat. No.
Hydrogen Peroxide, 50%	20 mL	490 mL	21196-49
Mineral Stabilizer	6 drops	50 mL SCDB	23766-26
Nesslers Reagent	2 mL	500 mL	21194-49
Polyvinyl Alcohol Dispersing Agent	6 drops	50 mL SCDB	23765-26
Potassium Hydroxide Standard Solution, 1.0 N	varies	50 mL SCDB	23144-26
Potassium Hydroxide Standard Solution, 8.0 N	varies	100 mL MDB	282-32
Sulfuric Acid, ACS, concentrated	6 mL	500 mL	979-49
TKN Indicator Solution	2 drops	50 mL SCDB	22519-26

REQUIRED EQUIPMENT AND SUPPLIES

Boiling Chips, silicon carbide	2-3	500 g	20557-34
Cots, finger	2	2/pkg	14647-02
DR/4000 1-Inch Cell Adapter	1	each	48190-00
Cylinder, graduated mixing, 25-mL	2	each	21190-40
Pipet, TenSette, 0.1 to 1.0 mL	1	each	19700-01
Pipet Tips, for 19700-01 TenSette Pipet	2	50/pkg	21856-96
Safety Shield, for Digesdahl	1	each	20974-00
Select one based on available voltage:			
Digesdahl Digestion Apparatus, 115 VAC	1	each	23130-20
Digesdahl Digestion Apparatus, 230 VAC	1	each	23130-21

OPTIONAL REAGENTS AND STANDARDS

Nitrogen Standard Solution, 1 mg/L NH_3–N	500 mL	1891-49
Ammonia Nitrogen Standard Solution, 100 mg/L NH_3–N	500 mL	24065-49
Nitrogen Standard Solution, 10-mL Voluette ampule, 150 mg/L NH_3–N	16/pkg	21284-10
Potassium Hydroxide, 12.0 N	500 mL	230-49

OPTIONAL EQUIPMENT AND SUPPLIES

Ampule Breaker Kit	each	21968-00
Bottle, glass dispenser, 118-mL	each	591-00
Bottle, plastic, wash, 1000-mL	each	620-16
Cylinder, graduated, 50-mL	each	508-41
DR/4000 Carousel Module Kit	each	48070-02
DR/4000 Flow Cell Module Kit, 1-inch	each	48070-04
DR/4000 Flow Cell Module Kit, 1-cm	each	48070-05
DR/4000 Sipper Module Kit, 1-inch	each	48090-03
Mini-Grinder, 120 VAC	each	20991-00
Pipet Filler	each	12189-00
Pipet, volumetric, Class A, 0.50-mL	each	14515-34
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 4.00-mL	each	14515-04
Pipet, volumetric, Class A, 5.00-mL	each	14515-37
Pipet, volumetric, Class A, 10.00-mL	each	14515-38
Safety Glasses, clear	each	18421-00

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