# DETERMINATION OF TOTAL KJELDAHL NITROGEN IN WATER USING THE SEAL AQ2 DISCRETE ANALYZER

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## 1. SCOPE AND APPLICATION

1.1. The Seal AQ2 Discrete Analyzer is used for the determination of total Kjeldahl Nitrogen (TKN) in ground, surface, and saline waters, and domestic and industrial wastes. The calibration range is from the method detection limit (MDL) 0.080 to 4.0 mg N/L. The range may be extended by dilution up to a limit of 10 mg N/L of organic N in the sample prior to digestion. The practical quantitation limit (PQL) is 0.20 mg N/L.

1.2. The tests covered by this SOP are W-TKN and W-TKN-F.

## 2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA method 351.2, Rev. 2.0 (1993) and Seal AQ2 method EPA-111-A Rev. 4.

2.2. Prior to analysis, digestion converts free ammonia and organic nitrogen compounds to ammonium sulfate (DEP SOP NU-091).

2.3. Ammonium reacts with salicylate and hypochlorite in a buffered, alkaline solution in the presence of sodium nitroferricyanide (pH = 12.4-12.7) to form the salicylic acid analog of indophenol blue. The blue-green color produced is measured at 660 nm.

2.4. Interferences: The working buffer eliminates precipitation of calcium and magnesium hydroxides. Samples that are turbid must be filtered prior to determination. High nitrate concentrations (>10 mg N/L) interfere with the TKN preparation procedure. The interference can be either negative or positive. For any samples with a known nitrate concentration of 10 mg N/L or higher the TKN result should be qualified with a J qualifier or the sample diluted sufficiently to decrease the nitrate concentration to below 10 mg N/L PRIOR to digestion.

## 3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation -

| Instrument Unique ID | Component | Manufacturer | Model |
|---|---|---|---|
| Seal AQ2 1 | Discrete Analyzer | SEAL Analytical | AQ2+ |

3.2. Equipment-

| Equipment | Manufacturer/Vendor[1] | Model/Catalog Number |
|---|---|---|
| Vortex Mixer | Corning | LSE |
| Analytical Balance | Mettler Toledo | MS204TS/00 |
| pH Meter | Thermo Scientific | Orion Star A211 |
| Stirrer/Hot Plate | Corning | PC-420D |
| Stirrer | Corning | PC-210 |
| Stir bars, Assorted | Fisher (SR) | 14-513-61; 14-513-58; S68069 |
| Drying Oven | ThermoScientific | Lindberg Blue M |
| Desiccator Cabinet | Fisherbrand | 08-645-6 |
| Adjustable Pipette, 100–1000 µL | Eppendorf/Fisher | Research Plus, 3123000063 |


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| | | |
|---|---|---|
| Adjustable Pipette, 1–10 mL | Eppendorf/Fisher | Research Plus, 3123000080 |
| Volumetric Flask, Class A, Assorted | Fisher (SR) | 10-210-5B; 10-212B; 10-212-5L; 10-208M |
| Glass Beaker, Assorted | Fisher (SR) | 02-540G; 02-540K; 02-555-20E; 20-540R |
| Filtering Flask | Fisher (SR) | 10-180F |
| Magnetic Filter Funnel, 47 mm | Fisher | NC0006472 |
| Glass Funnel | Fisher (SR) | 10-346-5B |
| Watch Glass, 15 cm | Fisher | 02-612F |
| **Or Equivalent** | | |
| [1]**Note:** (SR) designates equipment is typically ordered through the in-house stockroom | | |

3.3. Consumable and frequently replaced supplies-

| Supplies/Consumables | Vendor[1] | Catalog Number |
|---|---|---|
| Sample Cups, 1.2 mL | SEAL Analytical (SR) | 5385 |
| Sample Reagent Segments | SEAL Analytical (SR) | 5000 |
| Reagent Wedge Containers | SEAL Analytical | 5010 |
| Peristaltic Tubing | SEAL Analytical | 5140 |
| Roller Lubricant Pen | SEAL Analytical | AQLB |
| Roller and Wash Bath Cleaning Swabs | SEAL Analytical | AQCSW-20 |
| Pipette tips, Eppendorf, 1000 µL | Fisher (SR) | 05-403-43 |
| Pipette tips, Eppendorf, 10 mL | Fisher (SR) | 05-403-116 |
| Syringe, Polypropylene, 10 mL | Fisher (SR) | B303134 |
| Syringe Filters, Polypropylene, 25mm (0.45 µm pore size) | Fisher (SR) | 05-713-404A |
| Glass Fiber Filters w/o Binders, 47 mm, 0.7 µm | Millipore Sigma | APFF04700 |
| Sample Beakers, Polypropylene, Assorted | Fisher (SR) | 08-732-119; 08-732-120; 08-732-122 |
| Weigh Dish, Hexagonal, Assorted | Fisher (SR) | 02-202-102; 02-202-100 |
| Weigh Dish, Aluminum, 110 mm | Fisher (SR) | 08-732-100 |
| Reagent Bottles, Polyethylene, 1 L | Fisher (SR) | 05-719-88 |
| Reagent Bottles, Nalgene Amber, 1L | Fisher (SR) | 03-313-8F |
| Reagent Bottle, Amber, 125 mL | Fisher | 03-313-3C |
| Parafilm | Fisher (SR) | 13-374-12 |
| Kimwipes, Assorted | Fisher (SR) | 06-666A; 06-666C |
| Transfer Pipet, 5 mL | Fisher (SR) | 13-711-5AM |
| **Or Equivalent** | | |
| [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom | | |

## 4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 and NU-042)

**Note**: Before preparing reagents or standards, or running samples, read the safety notes in the safety/hazardous waste section of this SOP and the MSDS for the chemicals used in this analysis.

**Note**: Reagent and standard preparations are documented in the LIMS 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the prep worksheet in LIMS or in the Reagent and Standard


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Logsheet submitted with each analysis folder. If available, a Certificate of Analysis for each purchased standard, reagent, and the standard reference material is maintained in the REAGENT_PREP folder on the Nutrients drive. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

**Note**: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

4.1. Chemicals:

| | Chemical | Alias | Manufacturer[1] | Catalog Number | Purity | Expiration[2] | Storage |
|---|---|---|---|---|---|---|---|
| 4.1.1. | Ammonium Chloride | $NH_4Cl$ | Fisher Chemical | A661-500 | ACS, ≥99.5% | 5 years (*) | Room Temp |
| 4.1.2. | Glycine | $C_2H_5NO_2$ | Sigma Aldrich | G7126 | ≥99% | 6 years (*) | Desiccator |
| 4.1.3. | β-Glycerophosphoric acid disodium salt pentahydrate | Organic P, $C_3H_7Na_2O_6P$ *$5H_2O$ | Acros Organics | 410991000 | ≥98% | N/A | Room Temp |
| 4.1.4. | Complex Nutrients, WasteWatR | ALTQC, ERA | ERA | 525 | See COA | See COA | Room Temp |
| 4.1.5. | Sulfuric acid | $H_2SO_4$ | Fisher Chemical (SR) | A300S-212 | Concentrated, ACS Plus ≥95% | 5 years (*) | Room Temp |
| 4.1.6. | Sodium Hydroxide | NaOH | Fisher Chemical (SR) | S318-3 | ACS, ≥97% | 5 years (*) | Room Temp |
| 4.1.7. | Sodium Salicylate | $NaC_7H_5O_3$ | Sigma-Aldrich | S3007-500G | ≥99.5% | 4 years (*) | Room Temp |
| 4.1.8. | Sodium nitroferricyanide | $Na_2[Fe(CN)_5NO]$ *$2H_2O$ | Fisher Chemical | S350-100 | ACS, ≥99% | 5 years (*) | Room Temp |
| 4.1.9. | Sodium Hypochlorite, approx. 6% | NaOCl | Ricca | 4795.7-32 | 6.0% Chlorine | See COA | Fridge |
| 4.1.10. | Sodium potassium tartrate tetrahydrate | $NaKC_4H_4O_6$ * $4H_2O$ | Fisher Chemical (SR) | S386-500 | USP, ≥99% | 3 years (*) | Room Temp |
| 4.1.11. | Sodium phosphate dibasic heptahydrate | $HNa_2O_4P$ * $7H_2O$ | Fisher Chemical (SR) | S373-500 | ACS, ≥98% | 5 years (*) | Room Temp |
| 4.1.12. | Ethylenediamine tetraacetic acid disodium salt, EDTA | $C_{10}H_{14}N_2Na_2O_8$ *$2H_2O$, For cleaning | Fisher Chemical | S311-500 | ACS, ≥99% | 5 years (*) | Room Temp |
| 4.1.13. | Cuvette Cleaning Solution | For cleaning | SEAL Analytical | 5676 | N/A | N/A | Room Temp |
| 4.1.14. | Hellmanex III | For cleaning | Hellma | Z805939 | N/A | N/A | Room Temp |
| 4.1.15. | Hydrochloric Acid | HCl, For cleaning | Fisher Chemical (SR) | A144S-212 | Concentrated, ACS Plus | 5 years (*) | Room Temp |
| 4.1.16. | Bleach, Generic | NaOCl, For cleaning | Store Brand (no Clorox) | N/A | No additives, ~6% | N/A | Fridge |
| 4.1.17. | DI Water | $H_2O$ | In house | N/A | N/A | Daily | N/A |

**Or Equivalent**

[1]**Note:** (SR) designates chemical is typically ordered through the in-house stockroom

[2]**Note:** (*) designates manufacturer's suggested retest date. No expiration date given.



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**Note:** Wash all glassware and bottles with 1:1 HCl and rinse at least 7 times with DI water before preparing reagents and standards. Make sure any containers that are used to store prepared reagents and standards are clean and dry.

4.2. Reagents:

4.2.1. Stock Sodium Hydroxide, 20 % Solution –

| | |
|---|---|
| NaOH (4.1.6.) | 200 g |
| DI water (4.1.17.) | to 1000 mL |

In a 1 L volumetric flask containing approx. 700 mL of DI water, dissolve 200 g of NaOH. Solution will become hot. Let solution come to room temperature, then bring to volume with DI water.

Store at room temperature. Holding time, 180 days.

4.2.2. Stock Buffer Solution –

| | |
|---|---|
| Sodium phosphate dibasic heptahydrate (4.1.11.) | 254 g |
| NaOH (4.1.6.) | 20 g |
| DI water (4.1.17.) | to 1000 mL |

In a 1 L volumetric flask containing approx. 700 mL of DI water, add 254 g of sodium phosphate dibasic heptahydrate and 20 g of NaOH on a stirrer/hot plate. Stir until dissolved, approx. 60 minutes. Warm solution slightly if needed to dissolve completely. If heated, let solution come to room temperature, then bring to volume with DI water.

Prone to precipitation with cooler temperatures.

Store at room temperature. Holding time, 60 days.

4.2.3. Stock Sodium Potassium Tartrate, 10% Solution –

| | |
|---|---|
| Sodium potassium tartrate (4.1.10.) | 200 g |
| NaOH (4.1.6.) | 10-12 pellets |
| DI water (4.1.17.) | to 2000 mL |
| Sulfuric Acid (4.1.5.) | to pH 6-7 |

In a 2 L beaker containing approx. 1.2 L of DI water, dissolve 200 g of sodium potassium tartrate and 10-12 pellets of NaOH. While stirring, boil solution for 45 minutes to drive away any residual ammonia contamination. Cover with watch glass to cool. After solution has come to room temperature, transfer to 2 L volumetric flask and bring to volume with DI water. Invert to mix thoroughly.

Transfer solution to a clean 2 L beaker with a stir bar/plate. Using a calibrated pH meter (see NU-026), adjust the pH to that of DI water by adding concentrated sulfuric acid dropwise until desired pH (approx. pH 6-7) is reached.

Store in refrigerator. Holding time, 180 days.

4.2.4. Stock Color Solution –


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| | |
|---|---|
| Sodium nitroferricyanide (4.1.8.) | 3 g |
| DI water (4.1.17.) | to 100 mL |

In a 100 mL volumetric flask containing approx. 50 mL of DI water, dissolve 3 g of sodium nitroferricyanide. Bring to volume with DI water.

Transfer to amber bottle. Store in refrigerator. Holding time, 30 days.

Re-prepare if solution develops a blue-green tint.

**Caution:** Can produce cyanide gas if in contact with acid. Refer to safety section for handling and disposal (10.7.).

4.2.5. Stock Salicylate Solution –

| | |
|---|---|
| Sodium salicylate (4.1.7.) | 150 g |
| NaOH (4.1.6.) | 20 g |
| DI water (4.1.17.) | to 1000 mL |

In a 1 L volumetric flask containing approx. 500 mL of DI water, add 150 g of sodium salicylate using a glass funnel. Add 20 g of NaOH and stir until dissolved. Bring to volume with DI water and invert to mix thoroughly.

Using a vacuum filtration apparatus, filter solution with a glass fiber filter.

Transfer to amber bottle. Store in refrigerator. Holding time, 60 days.

4.2.6. Working Color Reagent –

| | |
|---|---|
| Stock Salicylate Solution (4.2.5.) | 40 mL |
| Stock Color Solution (4.2.4.) | 0.25 mL |

In a clean reagent wedge, pipet 40 mL of the Stock Salicylate Solution and 0.25 mL of the Stock Color Solution. Aspirate with pipette to mix thoroughly.

Store at room temperature. Holding time, 2 days.

For best results, prepare fresh daily.

4.2.7. Working Buffer Reagent –

| | |
|---|---|
| Stock Sodium Potassium Tartrate, 10% (4.2.3.) | 50 mL |
| Stock Buffer Solution (4.2.2.) | 20 mL |
| Stock Sodium Hydroxide, 20% (4.2.1.) | 18.4 mL |
| DI water (4.1.17.) | to 100 mL |

In a clean and empty 100 mL volumetric flask, add 50 mL of the Stock Sodium Potassium Tartrate 10% Solution, then 20 mL of the Stock Buffer Solution. **Mix together**, then add 18.4 mL of the Stock Sodium Hydroxide, 20% Solution. Bring to volume with DI water and invert to mix thoroughly.

**The order and steps in which the reagents are added is important!**

Store at room temperature. Holding time, 3 days.

For best results, prepare fresh daily.


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4.2.8. Working Hypochlorite Reagent –

| | |
|---|---|
| Sodium Hypochlorite, approx. 6.0% (4.1.9.) | 10.0 mL |
| DI water (4.1.17.) | to 50 mL |

In a 50 mL volumetric flask containing approx. 30 mL of DI water, add 10.0 mL of sodium hypochlorite. Bring to volume with DI water and invert to mix thoroughly.

Store at room temperature. Holding time, 3 days.

For best results, prepare fresh daily.

**Note:** Please check the hypochlorite concentration of stock solution and adjust the volume used as necessary.

4.2.9. Alkaline EDTA Cleaning Rinse (Control 1) –

| | |
|---|---|
| Sodium EDTA (4.1.12.) | 10 g |
| NaOH (4.1.6.) | 20 g |
| DI water (4.1.17.) | to 1000 mL |

In a 1 L volumetric flask containing approx. 600 mL of DI water, dissolve 10 g of sodium EDTA and 20 g NaOH. Dilute to volume with DI water and invert to mix.

Store at room temperature. Used for cleaning only.

**Note:** Safe for phosphate analysis.

4.2.10. Hellmanex Cleaning Solution –

| | |
|---|---|
| DI water (4.1.17.) | 40 mL |
| Hellmanex (4.1.14.) | 0.8 mL |

In a clean reagent wedge, pipet 40 mL of DI water and 0.8 mL of Hellmanex. Aspirate to mix.

Store at room temperature. Used for cleaning only.

**Caution:** Do not use if running phosphate analysis on same instrument.

4.3. Standards:

4.3.1. $NH_3$ stock standard, 1000 mg N/L –

| | |
|---|---|
| Ammonium chloride (4.1.1.) | 3.819 g |
| DI water (4.1.1.) | to 1000 mL |

Before preparation, dry at least 4 g of $NH_4Cl$ for 12-24 hours at 105°C. Cool to room temperature in desiccator after drying period finished.

In a 1 L volumetric flask containing approx. 800 mL of DI water, dissolve 3.819 g of dried $NH_4Cl$. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

**Note:** This solution is also used for ammonia analysis (see DEP SOP NU-104).


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4.3.2. TKN Working Calibration Standard (CAL STD, 4 mg N/L) –

| | |
|---|---|
| 1000 mg N/L $NH_3$ stock standard (4.3.1.) | 4.0 mL |
| Sulfuric acid (4.1.5.) | 2 mL |
| DI water (4.1.17.) | to 1000 mL |

In a 1 L volumetric flask containing approx. 600 mL of DI water, pipette 4.0 mL of the 1000 mg N/L $NH_3$ stock standard and **acidify** with 2 mL of sulfuric acid. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store at room temperature. Holding time, 30 days.

Digest according to DEP SOP NU-091.

4.3.3. TKN Continuing Calibration Verification (CCV) Standard (1.0 mg N/L) –

| | |
|---|---|
| 1000 mg N/L $NH_3$ stock standard (4.3.1.) | 1.0 mL |
| Sulfuric acid (4.1.5.) | 2 mL |
| DI water (4.1.17.) | to 1000 mL |

In a 1 L volumetric flask containing approx. 600 mL of DI water, pipette 1.0 mL of the 1000 mg N/L $NH_3$ stock standard and **acidify** with 2 mL of sulfuric acid. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store at room temperature. Holding time, 30 days.

Digest according to DEP SOP NU-091.

4.3.4. ALTQC Stock Standard (ERAQCXX Stock)–

The ALTQC stock standard is used as a second source reference material and must have a different supplier from the calibration standards. Currently, the second source supplier used is Environmental Resource Associates (ERA).

| | |
|---|---|
| Complex Nutrients Waste WatR (4.1.4.) | see ERA's instructions |
| DI water (4.1.17.) | see ERA's instructions |

The Complex Nutrients, WasteWatR is purchased as a concentrated ampule that must be diluted according to the manufacturer's instructions prior to analysis. The resulting prepared standard will be the **certified value** according to the manufacturer's certificate of analysis (COA).

Store in refrigerator. Holding time, 90 days.

4.3.4.1. ALTQC Working Standard (ERAQCXX Working) –

| | |
|---|---|
| ALTQC Stock Standard (4.3.4.) | refer to certified value |
| Sulfuric acid (4.1.5.) | 2 mL |
| DI water (4.1.17.) | to 1000 mL |

In a 1 L volumetric flask, add the appropriate amount of the ALTQC Stock Standard solution so that the working concentration is diluted within the calibration range. **Acidify** with 2 mL of sulfuric acid. Bring to volume with DI water and invert 12 times to mix thoroughly.


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Store in refrigerator. Holding time, 30 days.

Digest according to DEP NU-091.

4.3.5. TKN/TP Organic Spiking Solution (TKN: 150 mg N/L, TP: 50 mg P/L) –

| | |
|---|---|
| Glycine (4.1.2.) | 0.8040 g |
| β-glycerophosphoric acid (4.1.3.) | 0.4941 g |
| DI water (4.1.17.) | to 1000 mL |

Before preparation, dry at least 1.1 g of glycine overnight at 60 $^{o}$C. Cool to room temperature in desiccator after drying period finished.

In a 1 L volumetric flask containing approx. 700 mL of DI water, dissolve 0.8040 g of glycine and 0.4941 g of β-glycerophosphoric acid. **Do not acidify.** Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

**Note:** This solution is used to prepare lab fortified blanks (LFB), matrix spikes (MS) and matrix spike duplicates (MSD). It is also used for the analysis of TP and TKN in soils (DEP SOPS NU-084 and NU-101, respectively).

Glycine: $C_2H_5NO_2$, Formula wt: 75.07 g/mol.
Molecular weight factor = 14.0067/75.07 = 0.1866.

β-glycerophosphoric acid: $C_3O_{11}H_{17}PNa_2$, Formula wt: 306.11 g/mol.
Molecular weight factor = 30.9738/306.11 = 0.1012.

4.3.6. TKN/TP PQL standard (PQCK2) (TKN: 0.20 mg N/L, TP: 0.06 mg P/L) –

| | |
|---|---|
| 1000 mg N/L $NH_3$ stock standard (4.3.1.) | 200 µL |
| 1000 mg P/L TP stock standard (see NU-084) | 60 µL |
| Sulfuric Acid (4.1.5.) | 2 mL |
| DI water (4.1.17.) | to 1000 mL |

In a 1 L volumetric flask containing approx. 700 mL of DI water, pipet 200 µL of the 1000 mg N/L $NH_3$ stock standard and 60 µL of the 1000 mg P/L TP stock standard. **Acidify** with 2 mL of sulfuric acid. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

Digest this standard according to DEP SOP NU-091.

**Note:** This solution is also used for the analysis of TP and TKN in soils (DEP SOP NU-084 and NU-101, respectively).

## 5. SAMPLE HANDLING AND PREPARATION

Refer to DEP SOP NU-091 for preparation, sample preservation, and handling details.

## 6. SAMPLE ANALYSIS AND QUANTITATION (SEE DEP SOP NU-033)

**Note**: Digested solutions containing particulate matter should be filtered through a 0.45 µm filter


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prior to analysis on the AQ2 Discrete Analyzer and then redigested with dilution to confirm the result.

**Note:** Samples with organic N concentrations above 10 mg N/L must be diluted prior to digestion in order to report results without a J qualifier. (Subtract the ammonia concentration, if known, from the TKN result to calculate the organic N concentration). Add a comment on the run report to document this observation.

6.1. Initial Preparation:

6.1.1. Remove reagents from the refrigerator. Check the expiration dates on all standards and reagents. Discard expired standards and/or reagents and re-prepare if necessary.

6.1.2. Select digested trays to be analyzed and vortex samples and standards thoroughly. Record worksheet ID/tray number on the Reagent and Standard Logsheet.

6.2. Calibration:

6.2.1. The instrument is calibrated using the digested 4.0 mg N/L working CAL STD (4.3.2).

6.2.2. The diluent for TKN is the digested blank (see DEP SOP NU-091).

6.2.3. The auto calibration feature of the AQ2 is used in the analysis of TKN. The standards in the calibration are as follows (C2 to C6 are prepared by the instrument from the digested working calibration solution (4.3.2.)).

| STD | Conc. (mg N/L) | Source |
|---|---|---|
| C1 | 0.00 | Digested blank (6.2.2.) |
| C2 | 0.20 | Instrument dilution of C7 |
| C3 | 0.40 | Instrument dilution of C7 |
| C4 | 1.0 | Instrument dilution of C7 |
| C5 | 2.0 | Instrument dilution of C7 |
| C6 | 3.0 | Instrument dilution of C7 |
| C7 | 4.0 | Digested CAL STD (4.3.2) |

6.3. Reagents, Controls, and Stock Standard Loading:

6.3.1. Fill three separately labeled AQ2 reagent vessels with the following: Working Color (4.2.6.), Working Buffer (4.2.7.) and Working Hypochlorite (4.2.8.).

6.3.2. Reagent Location - The reagent tray, with plastic wedge-shaped containers is located on the right side of the injector arm. It holds all reagents and controls (CCV and blank) in the assigned locations. The buffer is in position 4, the color reagent in position 5, and the hypochlorite reagent is in position 6.

6.3.3. Controls - Fill one reagent vessel with the digested 1.0 mg N/L CCV


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(4.3.3.) and place it in position 10. Fill another vessel with digested blank (CCB, 6.2.2.) and place it in position 11.

6.4. Analysis Setup/Sequence setup:

6.4.1. Turn on the AQ2 Analyzer (large button on the right side at the back of the instrument) and the computer. To start the software double-click on the AQ2 Icon on the screen. The username is '**Instrument 1'** and the password is 'password'.

6.4.2. Proceed to the daily maintenance (Section 9) if it is the first run of day. Otherwise continue to 6.4.3.

6.4.3. Check that the DI water reservoir is full and empty the waste container. Replace all used reaction segments.

6.4.4. In the software, go to the 'Setting' menu and select maintenance. Zero the reaction segment counter.

6.4.5. Setup of the analytical run –

Click on the scheduling icon on the left side of the screen. Choose **Reagent Set 3**. Select a free tray and name the run using the format TKNYYYYMMDD00#K where the # refers to the first (# = 1), second (# = 2), etc runs of the day.

The sequence screen should appear. Starting at **line 3**, type the sample sequence in the 'Sample ID' column. The software will automatically schedule the CCV/CCB every ten cups on the run and thus do not need to be typed into the sequence.

Highlight the typed lines and select "Auto Fill" to fill the sequence numbers, and the **TKN** test. The test should appear in the requested test column to the right of the sample ID's.

Click the 'save' icon. Click on the 'print' icon to print the bench sheet.

A typical run sequence includes the following:

CCV (4.3.3.) – automatically scheduled
CCB (digested blank, 6.2.2.) – automatically scheduled
**ERAQC#** (ALTQC, 4.3.4.1.)
**PQCK2** (4.3.6.)
**MBLK**
**LFB**
**Spiked sample (MS)** (4.3.5.) and **spike duplicate (MSD)/sample duplicate**
**Samples**
CCV
CCB

**Note:** Multiple trays can be run on the same sequence using one calibration. Indicate on the report which tray the BLK, CCV, and calibration standard came from. Identify the tray QC in the sequence. The first ALTQC should be identified as the initial calibration


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verification (ICV). A failing ICV renders the calibration unusable.

6.4.6. Pouring the analytical run - Cup positions one and two are reserved for the calibration standards. Cup 1 is left empty. The TKN calibration solution (6.2.1.) is placed in cup two. Pour the samples according to the cup position on the bench sheet.

6.4.7. Double-click on the 'Run' icon on the left side of the screen. A dialog box will appear. Select the tray that contains the sequence to be analyzed. Click 'OK'. Another dialog box will appear. Check the auto-standardize box and click 'OK'. (If the analysis should use the previous calibration do not check the auto-standardize option.) Select 'OK' on the following dialog box.

6.4.8. The reagent levels are checked by the instrument and are displayed on the upper right corner of your screen. If the levels are sufficient for the entire run the analysis will start; if not, the run will stop and display the reagent which has insufficient volume. Fill up the reagent and re-start the run according to 6.4.7.

6.5. Quantitation/Calculations:

6.5.1. The instrument will give the analysis results for TKN in mg N/L.

6.5.2. The QC-Manager calculates recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and spikes. For general calculations including RPDs and spike recoveries see Section the FDEP Quality Manual.

6.5.3. The high calibration standard is prepared according to section 4.3.2. The other standards used in the second order calibration are auto-diluted by the instrument from the high standard. The percent error of the standards and correlation coefficient are calculated by the instrument software. (See 8.5.)

6.6. Post-Run Procedure:

6.6.1. After the run has finished, print the AQ2 Test Parameters Report from the 'Settings' menu on the top of the screen.

6.6.2. Click on the 'Acceptance' menu located on the left side of the screen.

6.6.3. Click on the 'Accept All' Icon. This adds 'ACC' to the results. A pop-up window appears where the file name needs to be entered. Follow the naming convention described in 6.4.5. Accepting the run in the AQ2 creates the excel transfer file for uploading the data to the Laboratory Information Management System (LIMS). This file should be saved in the current year's subfolder located in \\fldep1\dear\labs\lab3\chem_data\Inorganic\Nutrients\AQ2\TKN_COP\.

6.6.4. Click on the 'Reporting' icon on the left side of the screen. Check the reporting format 'TKN/TP' and print the run report. It contains the calibration and all the data. Select the chronological order report function to print your report.


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6.6.5. Convert the excel file into Raw data in the QC Manager. The Instrument used is 'Nutrients: Seal AQ2 1'

## 7. DATA ARCHIVAL

7.1. The raw data is archived in paper format. A report folder is created for each analytical run. Occasionally more than one run may be in one folder. The folder is labeled with the test (W-TKN(-F)), filename/s (TKNYYYYMMDD001K), and initials of the analyst.

7.2. Each report folder should contain the following items - run report including raw data, calibration curve, method parameters, bench sheet, Reagent and Standard Logsheet, digestion temperature data logger report, and a copy of the data and QC assessments uploaded from QC Manager to LIMS at the time of test authorization. The sample preparation worksheet is archived electronically in the Prep Worksheet Manager module in the LIMS system.

7.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

7.4. AQ2 instrument data excel transfer files for upload to QC manager are stored in electronic format in the current year's subfolder located in: \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\AQ2\TKN_COP\.

7.5. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\fldep1\dear\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

## 8. QUALITY CONTROL/DATA ASSESSMENT (SEE NU-038 AND NU-043)

8.1. For method performance and acceptance criteria for QC measures and corrective action for out-of-control data and contingencies for handling out-of-control or unacceptable data refer to DEP SOPS NU-038 and NU-043.

8.2. A CCV (4.3.3.) and a CCB (digested blank, DEP SOP NU-091) are analyzed every 10 samples and at the beginning and end of each run. Recovery acceptance limits for the method blanks (MBLKs) and CCBs are < 0.080 and > -0.080 mg N/L. The check standard (CCV) recovery limits are 90 – 110 %

8.3. Each tray of digested samples contains a digested PQL level standard (PQCK2, 4.3.6.) and a certified standard reference material (ALTQC, 4.3.4.). Recovery acceptance limits are 70 -130 % for PQCK2 and 90 – 110 % for the ALTQC. The PQCK2 is also used for the ongoing MDL and PQL verification.

8.4. An MBLK, an LFB, a duplicate sample and/or duplicate matrix spikes are digested and analyzed with every batch of 10 or fewer samples (see DEP SOP NU-091). Acceptance limits for the LFB and matrix spike recoveries are 90 – 110 %. The RPD of sample/matrix spike duplicates must be less than 20 % for results above the PQL.


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8.5. Linear Calibration Range (LCR) - See FDEP Quality Manual, Appendix B, Section 7.0, 'Protocol for Determining the Test Method Range of Applicability'.

- The correlation coefficient of the second order calibration must be larger than 0.995.
- Acceptance limits for the blank level calibration standard (S1/S0) calculated results are <0.080 and > -0.080.
- Acceptance limits for the percent error of the PQL level calibration standard is ±30%.
- Acceptance limits for the percent error of calibration standards above the PQL are ±10%.
- Every day samples are analyzed a new calibration must be performed and verified at the beginning of the analysis. Consecutive runs on one day may use the same calibration.

## 9. MAINTENANCE/TROUBLESHOOTING

9.1. Empty and rinse the DI water reservoir at least three times. Refill to the top.

9.2. Empty the instrument rinse waste. Check that the instrument hazardous waste bottle isn't full.

9.3. In the SEAL AQ2 Program, go to 'Settings' then 'Maintenance.'

9.4. In 'Initialise,' press 'Zero Segments.' **Do not press 'OK' yet.**

9.4.1. Check to see if there are enough reaction segments from the day before to do maintenance. 2-3 unused is enough. Then press 'Cancel.' The probe will go to the first clean segment instead of Segment 1, spot 1.

9.4.2. If there are not enough clean segments, replace the segments. Double tap clean segments on the bench to knock out excess manufacturing particles, then place in the instrument. Press 'OK' to reset the used segments. The probe will go to Segment 1, spot 1.

9.5. Go to 'Diluter,' set the number of primes to 10, then press 'Start Primes.'

9.5.1. Watch the syringe piston to see if there are excess bubbles dragging from the top. A worn syringe piston can interfere with the calibration auto-dilution and analysis. Replace if necessary.

9.6. Clean the aspiration bath with a Q-tip or cleaning swap.

9.6.1. The particles from manufacturing can get caught in the cuvette. Daily cleaning of the aspiration bath helps prevent this from occurring.

9.7. In a reagent wedge in position 1, place either the Cuvette cleaning solution (4.1.13.), Hellmanex Cleaning Solution (4.2.10.) or the Alkaline EDTA Rinse (4.2.9.). Go to 'Cuvette Functions,' then press 'Extra Wash.'

9.7.1. Watch the glass sampler probe cleaner. A water vortex should be visible when the sampler probe is being washed and liquid should not drip out.


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9.7.2. Contamination between samples and reagents can occur if the probe cleaner is not operating properly. Liquid drips out of the glass probe cleaner when the probe creates a wider hole at the bottom of the glass (will look oval). This can occur over time or if the sampler probe is bent. Replace the probe cleaner and sampler probe if necessary.

9.8. After 'Extra Wash' is done, set the number of 'Auto Wash' to 5. Then press 'Auto Wash.'

9.8.1. Make sure the aspiration bath is filling and emptying each time. Change the two peristaltic tubing going to the aspiration bath if it is not.

9.9. After 'Auto Wash' is done, press 'Extra Wash' again.

9.10. After the second 'Extra Wash' is done, press the 'Auto Wash' again.

9.11. To adjust the aspiration, remove the cover from the top of the cuvette/lamp. Press 'Test Aspiration' and observe where the water line begins. It should be around 2 inches out of the cuvette. Repeat 'Test Aspiration' 2 more times, then check that the $4^{th}$, $5^{th}$, and $6^{th}$ repetition are in the same spot.

9.11.1. To make the water line come out of the cuvette further, increase the number of steps. Decrease the number of steps if it is too far out. Then repeat the above test.

9.11.2. If the water line is not around the same location in each repetition, random errors may occur in analysis. Replace the peristaltic tubing on the pump located next to the lamp.

9.12. In 'Initialise,' press 'Zero Segments,' and replace all used segments. Then press 'OK.'

9.13. Close the maintenance window then go to 'Settings' → 'Daily Startup' → 'Continue.' Write down the highest voltage and the corresponding filter in the maintenance book.

9.13.1. The highest voltage filter should remain the same for each instrument. A drastic change in voltage from day to day indicates a lamp going bad or an electrical issue.

## 10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents).

10.2. Safety glasses, gloves and lab coats should be worn at all times whilst handling samples and reagents.

10.3. Read the MSDS information for safe reagent and sample handling.

10.4. The solution used to digest samples contains copper, which is a hazardous waste and toxic. All waste from the instrument, digested samples, and standards made with digestion solution should be collected in bottles clearly labeled as hazardous waste. The waste should be disposed as acidic metals waste.


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10.5. Once instrument waste containing copper is filled and removed from the instrument it can be stored for no longer than three working days within the hazardous waste storage of the analytical laboratory area.

10.6. Rinse waste from the instrument that can be disposed of in the sink is to be accompanied by tap water.

10.7. The sodium nitroferricyanide (4.1.8.) used to prepare the color reagent produces cyanide gas if it encounters acid. Wear all personal PPE when preparing and thoroughly rinse flasks and containers after acid washing. Dispose excess sodium nitroprusside crystals by lab packing. Excess color reagent can be disposed of down the sink when flushed thoroughly with water.

## 11. REFERENCES

**For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.**

11.1. EPA Method 351.2, Rev. 2.0 (1993).

11.2. Seal AQ2 Method EPA-111-A, Rev. 4, Total Kjeldahl Nitrogen-N (Copper Catalyst) in Drinking, Ground and Surface Waters, Domestic and Industrial Wastes.

11.3. DEP SOP NU-033, Summary of Analysis Procedure for the General Chemistry and Nutrients Workgroups.

11.4. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

11.5. DEP SOP NU-041, Reagent Preparation Procedure.

11.6. DEP SOP NU-042, Standard Preparation Procedure.

11.7. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines.

11.8. DEP SOP NU-091, Preparation of Waters for Total Kjeldahl Nitrogen Analysis (Copper Catalyst).

11.9. DEP SOP NU-101, Determination of Total Kjeldahl Nitrogen in Soils using the SEAL AQ2 Discrete Analyzer.

11.10. DEP SOP NU-084, Determination of Total Phosphorus in Soils using the AQ2 Discrete Analyzer.

11.11. DEP SOP NU-104, Determination of Ammonia in Water using the AA3 Gas Diffusion Segmented Flow Analyzer.

11.12. DEP SOP LB-006, Standard Operating Procedure for Records Maintenance and Storage.

11.13. Quality Manual, Bureau of Laboratories, FDEP.



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11.14. Laboratory Health and Safety Plan.

## Appendix of Significant Changes

10/20/2009 Some minor corrections were made in section 4.

08/20/2010 Procedural clarifications some corrections mainly to section 4 and 6.

08/09/2011 Preparations of the PQL standard and spiking TKN solution were modified and now include phosphorous (these solutions also used for TKN and TP analysis in soils).

12/10/2019 Clarified Section 3. Added procedures for preparation of Alkaline EDTA Rinse and Hellmanex Cleaning Solution to Section 4. Section 6 was modified to clarify running multiple trays on one sequence. Added ongoing MDL/PQL verification information and acceptance limits for calibration standards recoveries to Section 8. Section 9 maintenance and troubleshooting was added. Section 10 safety and waste disposal procedure was clarified.

12/22/2020 In Section 3, the instrumentation, equipment, and supplies were updated and inputted into table format. In Section 4, chemicals were updated and inputted into table format. Preparation procedures for reagents and standards were reformatted and clarified. The calibration curve blank level standard limits were added to Section 8.


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