PREPARATION OF WATERS FOR TOTAL KJELDAHL NITROGEN ANALYSIS (COPPER CATALYST)

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1. SCOPE AND APPLICATION

1.1. This method details the digestion of waters for Total Kjeldahl Nitrogen (TKN) analysis. Digestates produced by this method are suitable for analysis by the Seal AQ2 Discrete Analyzer (DEP SOP NU-092) and the SEAL AA500 Flow Analyzer (DEP SOP NU-108). For analytical details such as calibration range and the method detection limit (MDL) see DEP SOP NU-092 and DEP SOP NU-108.

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

1.3. Applicable matrices are surface and saline water, and domestic and industrial wastewater.

2. SUMMARY OF THE METHOD

2.1. This SOP is based on EPA method number 351.2, Rev. 2.0 (1993) and AQ2 method No: EPA-111-A Rev.4

2.2. A digestion solution containing potassium sulfate, sulfuric acid and copper sulfate catalyst is added to the samples. The prepared samples are heated in a digestion block to convert organic nitrogen to its inorganic form (NH_4^+). The potassium sulfate raises the boiling temperature of the digestion and increases the rate of conversion.

2.3. Interferences: Nitrate in concentrations greater than 10 mg N/L can interfere with the digestion of TKN. 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 must be reported 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. Equipment:

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Digestion Block	SEAL Analytical	BD50
Type K Thermocouple	Omega Engineering	TJ72-CAXL-316U
Temperature Data Logger	Fisher Scientific	15-059-299
Male Connector	Omega Engineering	NC1658189
Vortex Mixer	Corning	LSE
Analytical Balance	Mettler Toledo	MS204TS/00
Stirrer/Hot Plate	Corning	PC-420D
Stir bar	Fisher Scientific (SR)	14-513-61
Drying Oven	ThermoScientific	Lindberg Blue M
Desiccator Cabinet	Fisherbrand	08-645-6
Adjustable Pipette, 10–100 µL	Eppendorf/Fisher	Research Plus, 3123000047
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher	Research Plus, 3123000063
Adjustable Pipette, 2-20 mL	Rainin	LTS L-20MLXLS, 17011788
Bottletop Dispenser, 0.5-5 mL	BrandTech	Dispensette S, 4600130
Bottletop Dispenser, 2.5-25 mL	Eppendorf/Fisher	Varispenser 2, 4966000045
Bottletop Dispenser, 2.5-25 mL	Corning/Fisher	07-201-131
Digestion Tubes, 75 mL	SEAL Analytical	4003005

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Digestion Beads, 0.25”	Saint-Gobain	09-191-30B
Poxygrid Test Tube Racks	Bel-Art	F18764-0001, F18762-0001
Volumetric Flask, Class A, Assorted	Fisher (SR)	10-212B; 10-212E; 10-212-5L; 10-208M
Glass Beaker, Assorted	Fisher (SR)	02-540G; 02-540K; 02-540M; 02-555-20E; 20-540R
Graduated Cylinder, Class A	Fisher (SR)	08-564-A
Water Bath	Fisherbrand	Isotemp GPD 10
Or Equivalent [1]Note: (SR) designates equipment is typically ordered through the in-house stockroom		

3.2 Consumables and frequently replaced supplies:

Supplies/Consumables	Vendor[1]	Catalog Number
Pipette tips, Eppendorf, 200 µL	Fisher (SR)	05-403-41
Pipette tips, Eppendorf, 1000 µL	Fisher (SR)	05-403-43
Pipette tips, Rainin, 20 mL	Rainin (SR)	17001131
Sample Beakers, Polypropylene, Assorted	Fisher (SR)	08-732-119; 08-732-120; 08-732-122
Kimwipes, Assorted	Fisher (SR)	06-666A; 06-666C
Weigh Dish, Hexagonal	Fisher (SR)	02-202-102
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Parafilm	Fisher (SR)	13-374-12
Aluminum Foil	Fisher (SR)	01-213-105
Or Equivalent [1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

Note: Each data logger and thermocouple combination is calibrated annually as a system, thus the thermocouples and data loggers cannot be interchanged. One USB interface cable allows all data logger devices to be connected to a PC running the Fisher Scientific Datalogger software to start and stop recording, download temperature data, etc.

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

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.

Note: All reagent and standard preparations must be 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. If available, a Certificate of Analysis for each purchased reagent or standard is also maintained in the REAGENT_PREP folder on the Nutrients drive at\\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TKN\CertificatesofAnalysis\ . All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: Prior to using the chemicals in this analysis, read their MSDS sheet and the safety notes found in Section 9 of this SOP.

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4.1. Chemicals:

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Storage
4.1.1.	DI water	H_2O	In house	N/A	N/A	N/A
4.1.2.	Potassium sulfate	K_2SO_4	Fisher Chemical (SR)	P305-500	ACS, ≥99%	Room Temp
4.1.3.	Cupric sulfate pentahydrate	$CuSO_4$ *$5H_2O$	Fisher Chemical	C493-500	ACS, ≥98%	Room Temp
4.1.4.	Sulfuric acid	H_2SO_4	Fisher Chemical (SR)	A300S-212	Concentrated, ACS Plus ≥95%	Room Temp
4.1.5.	Ammonium Chloride	NH_4Cl	Fisher Chemical	A661-500	ACS, ≥99.5 %	Room Temp
4.1.6.	Glycine	$C_2H_5NO_2$	Sigma Aldrich	G7126	≥99%	Desiccator
4.1.7.	β-Glycerophosphoric acid disodium salt pentahydrate	Organic P, $C_3H_7Na_2O_6P$ *$5H_2O$	Acros Organics	4100991000	≥98%	Room Temp
4.1.8.	Complex Nutrients, WasteWatR	ALTQC, ERA	ERA	525	See CoA	Room Temp/Fridge
4.1.9.	Hydrochloric Acid	HCl, For cleaning	Fisher Chemical (SR)	A144S-212	Concentrated, ACS Plus	Room Temp
4.1.10.	Nitric Acid	HNO_3, For cleaning	Fisher Chemical (SR)	A509P-212	Concentrated, Trace Metal	Room Temp
4.1.11.	Ottawa Sand	SiO_2, For probes	Fisher Chemical (SR)	S23-3	N/A	Room Temp
Or Equivalent [1]**Note:** (SR) designates chemical is typically ordered through the in-house stockroom						

Deionized water is replaced daily. All other purchased chemicals are given the expiration date from the CoA and/or chemical container. If there is not an expiration date listed, the expiration date is designated by following the protocol in SOPs NU-041 and NU-042.

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. Diluent –

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Sulfuric acid (4.1.4.)	4 mL
DI water (4.1.1.)	to 2000 mL

In a 2 L volumetric flask containing approx. 1.8 L of DI water, pipette 4 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.

4.2.2. Copper Sulfate Digestion Solution –

Sulfuric acid (4.1.4.)	268 mL
Potassium sulfate (4.1.2.)	268 g
Cupric sulfate pentahydrate (4.1.3.)	22.8 g
DI water (4.1.1.)	to 2000 mL

Place a 2 L beaker filled with approx. 1.2 L of DI water and a stir bar on a stir plate in a fume hood. Slowly add 268 mL of sulfuric acid while stirring. Then dissolve 268 g of potassium sulfate and 22.8 g of copper sulfate. Let solution stir until it reaches room temperature, then transfer to a 2 L volumetric flask. Bring to volume with DI water and invert 12 times to mix thoroughly.

Caution: This solution will become hot. If needed, cover with parafilm after letting cool slightly and let come to room temperature overnight. Make sure that the beaker is clearly labeled.

Transfer to clean glass bottle. Store at room temperature. Holding time, 30 days.

4.3. Standards and spiking solution:

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

Ammonium chloride (4.1.5.)	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 105ºC. Cool to room temperature in desiccator after drying.

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).

4.3.2. TKN PQL standard (PQCK2, 0.20 mg N/L) –

1000 mg N/L NH_3 stock standard (4.3.1.)	200 µL
Sulfuric Acid (4.1.4.)	2 mL
DI water (4.1.1.)	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. **Acidify** with 2 mL of concentrated 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.

4.3.3. TKN Organic Spiking Solution (225 mg N/L) –

Glycine (4.1.6.)	1.206 g
DI water (4.1.1.)	to 1000 mL

Before preparation, dry at least 2 g of glycine overnight at 60 ºC. Cool to room temperature in desiccator after the drying period is finished.

In a 1 L volumetric flask containing approx. 700 mL of DI water, dissolve 1.206 g of glycine. **Do not acidify.** Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

Note: For convenience, a combined TKN/TP spiking solution (TKN: 150 mg N/L; TP: 50 mg P/L) can be prepared by adding 0.4941 g β-glycerophosphoric acid (4.1.7.) to the flask prior to bringing the standard to volume (see DEP SOP NU-049 for details). If a combined spiking standard is made, this standard should be named "TKN/TP ORG SPIKE" in the Standard Preparation Tracker.

Note: This solution is used to prepare lab fortified blanks (LFB), matrix spikes (MS) and matrix spike duplicates (MSD). The combined TKN/TP spiking solution 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.

4.3.4. ALTQC Stock Standard (ERAQCXX Stock) –

Complex Nutrients Waste WatR (4.1.8.)	see ERA's instructions
DI water (4.1.1.)	see ERA's instructions

The ALTQC standard is used as a second source standard and must have a different supplier or a different lot number than the calibration standards. A certified reference standard is preferred. Currently, the second source supplier used is Environmental Resource Associates (ERA).

The Complex Nutrients, WasteWatR is purchased as a concentrated ampule that must be diluted according to the manufacturer's instructions to produce a standard of the certified concentration.

Store in refrigerator. Holding time, 90 days.

4.3.5. ALTQC Working Standard (ERAQCXX Working) –

ALTQC Stock Standard (4.3.4.)	refer to certified value
Sulfuric Acid (4.1.4.)	2 mL
DI water (4.1.1.)	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 to approximately

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the middle of the calibration range. **Acidify** with 2 mL of concentrated sulfuric acid. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

Note: In cases where the certified value of the quality control standard (4.1.8.) falls within the calibration range, further dilution is not necessary. However, the ALTQC prepared MUST be acidified to 0.2% H_2SO_4 and would be considered the ALTQC Working Standard (4.3.5.). Make sure a 30 day holding time is associated to this standard.

4.3.6. TKN Working Calibration Standard (CAL STD, 4.0 mg N/L) –

1000 mg N/L NH_3 stock standard (4.3.1.)	4.0 mL
Sulfuric Acid (4.1.4.)	2 mL
DI water (4.1.1.)	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 concentrated sulfuric acid. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

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

1000 mg N/L NH_3 stock standard (4.3.1.)	1.0 mL
Sulfuric Acid (4.1.4.)	2 mL
DI water (4.1.1.)	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 concentrated sulfuric acid. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

4.3.8. TKN MDL Verification Spike Standard (MDL SPK, 0.10 mg N/L) –

1000 mg N/L NH_3 stock standard (4.3.1.)	200 µL
Sulfuric Acid (4.1.4.)	2 mL
DI water (4.1.1.)	to 1000 mL

In a 1 L volumetric flask containing approx. 700 mL of DI water, pipet 100 µL of the 1000 mg N/L NH_3 stock standard. **Acidify** with 2 mL of concentrated sulfuric acid. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

5. SAMPLE PRESERVATION, PREPARATION AND HANDLING (SEE DEP SOP NU-033)

5.1. Aqueous samples are preserved at collection with sulfuric acid to a pH of less than 2 units (0.2 % sulfuric acid). They are stored upon receipt in the Nutrient refrigerator located in the receiving room at ≤ 6 °C (not frozen) and have a 28-day holding time.

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5.2. A batch may contain up to 10 samples. Fresh and ground water samples may be batched together. Effluents and influents are batched separately.

5.3. All samples having conductivities above 5000 µmhos/cm (equivalent to 3.0 ppt salinity) are batched separately. If a sample conductivity exceeds 5000 µmhos/cm it should be digested in a salt batch even if it is not labeled as a salt matrix.

5.3. Samples with organic N concentrations above 10 mg N/L must be diluted prior to digestion 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.

5.4. Remove the samples from the refrigerator following the proper protocols to document the sample chain of custody. Allow samples to come to room temperature before digestion. Begin heating the digestion block to 180 °C using Program 1 on the block controller (see Section 5.5.13. below).

5.5. Preparation Procedure for Water Samples:

Note: While preparing the samples, any extra sample waste not containing copper can be discarded down the sink. Any samples/solutions containing digestion solution that need to be discarded are to be collected in a hazardous waste container (see Section 9.4.).

5.5.1. Check the compliance of the refrigerator following the instructions in NU-REFTEMPGUIDE and NU-REFTEMPCHK before opening door, if not already checked for the day. Remove the following standard solutions from the refrigerator: spiking solution (4.3.3.), PQL solution (4.3.2.), ALTQC solution (4.3.5.), CAL STD (4.3.6.), and the CCV check solution (4.3.7.), and MDL SPK solution (4.3.8.). Allow standards to come to room temperature before pouring them out for digestion.

5.5.2. For each tray one MDL SPK, PQCK2, ALTQC, CAL STD and CCV check standard should accompany the batches. For every batch digested, a blank (MBLK; made with diluent, 4.2.1.), an LFB, a matrix spike (MS) and a duplicate sample and/or duplicate matrix spike (MSD) are required.

Note: An MDL SPK should be prepared with every tray, if possible. At minimum, an MDL SPK must be prepared twice a quarter for yearly statistics calculations, as required by our Quality Manual.

5.5.3. Create a new prep worksheet in the Prep Worksheet Manager module in LIMS.

5.5.3.1 Open the Prep Worksheet Manager module in LIMS and choose the first icon (which looks like a blank page) to create a new worksheet. Choose “351.2 (March 1983)” from the list of available preparation IDs, select the sample IDs to be added to the worksheet, assign the worksheet to the correct analyst, and click the checkmark to create the worksheet. The worksheet ID generated in LIMS will be the tray number for the digestion.

5.5.3.2. Open the new worksheet by clicking the spreadsheet icon in the Prep Worksheet Manager module and double-clicking the appropriate

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worksheet ID from the list. Complete the header information for the sheet: SOP (NU-091), prep analyst name, prep start date/time and prep completion date/time, equipment ID (e.g., digestion block 'Neptune', 'Hercules', or 'Atlas'), probe position, pipettes and dispensers used, and the reagents and spike standard solution serial numbers (ensure that the corresponding identities and expiration dates are correct by comparing to the labels on the bottles and equipment). The prep start time should be reflected by the initial time on the datalogger upon activation, while the completion time should be reflected by the time of the final data point that the datalogger obtains.

5.5.3.3. Update the sample table, beginning with the starter rows for QC samples digested on the tray, ensure that serial numbers, identities, and expiration dates for the standard solutions used are correct. A single prep batch ID will be generated automatically for every sample on the sheet; therefore, assign other prep batch IDs, as necessary, by 'Control+Clicking' the sample IDs, MBLK, and LFB to be batched together and generating a new prep batch ID for each batch. For the matrix spike sample in each batch, duplicate its row in the sample table **twice** by right-clicking on the row and selecting "Duplicate Row", then add the spike code '_O3_' and the letter A or B as a suffix to the sample ID. Save the updates by clicking the "Save" button at the bottom of the worksheet.

5.5.4. Dry the outside of the clean tubes, if needed. If no clean tubes are available, clean the number of tubes needed before proceeding. The detailed procedure for cleaning tubes is given in DEP SOP NU-046.

5.5.5. Label the tubes using a black or red permanent marker following the batching order on the digestion worksheet. The worksheet ID/tray number is written on the first tube on the tray.

Note: Tube markings made with a black or red marker are known to survive the harsh conditions of the digestion. Markers of other colors may not produce stable enough markings.

5.5.6. If the tubes were cleaned manually, pour out any excess water in the clean tubes. Rapidly invert each sample, and then rinse each tube with 1-2 mL of sample. For dishwasher-cleaned tubes this practice usually is not required.

5.5.7. Invert each sample multiple times until all particulate matter is suspended and then pipette 25 mL into the respective tubes. The calibration standard, check standards, ALTQC, MBLKs, and LFBs are prepared the same way. Two extra tubes with diluent solution are also prepared as blanks, for use as CCBs and to make dilutions on the analytical run. The diluent for MBLKs, LFBs and CCBs can also be added using calibrated bottletop dispensers.

If a sample requires dilution, the dilution can be prepared either in a digestion tube or in a volumetric flask. Indicate the dilution factor on the digestion tube. Use the following table for dilutions made directly in the tube.

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Note: For the preparation of all dilutions use the diluent, 0.2 % H_2SO_4 (4.2.1.).

Dilution	Pipette Sample Volume (mL)	Pipette Diluent Volume (mL)	Total Volume in Tube (mL)
1:2	12.5	12.5	25
1:5	5.00	20.0	25
1:10	2.50	22.5	25
1:25	1.00	24.0	25
1:50	0.50	24.5	25

For dilutions that are 100 times or greater, prepare in a volumetric flask by pipetting the appropriate amount of sample into the flask first and then bring the volume up to the mark with diluent (4.2.1). Transfer 25 mL of the diluted sample into the digestion tube. Add a note in the comments section on the digestion worksheet with the exact dilution made in the volumetric flask.

Record dilution factors and actual sample volume used on the prep worksheet.

Note: NEVER dilute Equipment Blanks, Field Blanks, Trip Blanks, or DI Water samples unless told to do so by a supervisor.

5.5.8. Spiking procedure (MS, MSD, LFB) – The spike level is 1.8 mg N/L. The spikes are prepared by adding 200 µL of 225 mg N/L TKN spiking solution (4.3.3.) to a 25 mL aliquot of sample (diluted if needed) and to a 25 mL aliquot of diluent (4.2.1.) for the LFBs.

5.5.9. Add 2 clean boiling beads to each tube. Do not add more than 3 beads to water samples.

5.5.10. Add 5 mL of the copper sulfate digestion solution (4.2.2.) to each tube.

5.5.11. Place the tubes in the digestion block.

5.5.12. One spot on the block is chosen to place the thermocouple of the data logger system (3.1.). This system is used for temperature recording during the digestion process. The data logger program is started in advance using the Fisher Scientific Datalogger software, as follows:

5.5.12.1. Plug data logger into the USB cable at the PC with the software installed.

5.5.12.2. Open the software. The data logger ID will appear in the “Connected Devices” panel of the software interface. Click on the ID once to highlight it.

5.5.12.3. In the Device tab menu bar, click Quick Start (this will use the most recent device settings, visible in the Connected Devices panel). When the data logger has been properly started, the status will read “Running”. It is recommended that data collection rate is set to 1 point every 5 minutes.

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5.5.12.4. Unplug the data logger from the USB interface cable, and attach to the appropriate thermocouple cable outside the hood where the digestion block sits.

5.5.13. Lower the hood to set mark. Select and execute the block digestion program.

5.5.13.1. If the block was preheated to 180 °C using program 1, continue with program 2.

5.5.13.2. If the block was not preheated at the time the tubes were placed in the block start the digestion by selecting program 2, which includes an initial heating phase at 180°C. These initial heating stages are necessary to hydrolyze the organic nitrogen and drive off most of the water. Watch the samples to be sure they do not "bump" out of the tubes.

Note: **The method requirement for complete digestion is a minimum of 30 min at 380 °C.** Block temperatures above 395 °C should be avoided due to the possibility of side reactions. Other block programs may be used provided they meet the temperature requirements. A list of programs for each block can be found in the table below. The block controller settings may be adjusted depending upon the actual temperature recorded by the data logger-thermocouple system; block temperatures should be verified whenever the controller settings are adjusted and documented in the block maintenance manuals located in B411.

Program Name	Program Description	Program Steps
Program 1	"Preheat" – This program preheats the block to 180°C. It is used in conjunction with Program to reduce the time of the digestion. **This program is not an analytical program and is used prior to the tray being placed on the block.**	1. Ramp 180 °C, 5 °/min 2. Hold for 400 min 3. End Program
Program 2	"Water" – This program is the standard heating cycle for TKN_D. The initial heating stage is necessary to hydrolyze the organic nitrogen and drive off most of the water.	1. Step to 180 °C 2. Hold for 60 min 3. Ramp 380 °C, 5 °/min 4. Hold for 60 min 5. End Program
Program 3	"Overnight Cook" – This program digests the tray overnight. It is used only when the analyst is unable to reconstitute the tray the same day. The program holds the tray at 50 °C after digestion to facilitate sample mixing after reconstituting.	1. Step to 50 °C 2. Hold for 30 min 3. Ramp 80 °C, 1 °/min 4. Hold for 75 min 5. Ramp 110 °C, 1 °/min 6. Hold for 90 min 7. Step to 140 °C 8. Hold for 90 min 9. Step to 180 °C 10. Hold for 60 min 11. Step to 380 °C 12. Hold for 60 min 13. Step to 50 °C 14. Hold for 450 min 15.End Program

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Program 5	"Fast Recook" – This program is used when the first digestion did not reach temperature. The initial heating stage is not needed, since it was performed during the first digestion. **This program should not be used as the initial digestion.**	1. Step to 380 °C 2. Hold for 55 min 3. End Program
Program 6	"Fast Reheat" – This program reheats the tray to facilitate sample mixing during reconstituting. **This program is used PRIOR to reconstituting.**	1. Step to 90 °C 2. Hold for 15 min 3. End Program
Program 7	"Overnight Recook" – This program is used when the first digestion did not reach temperature and the analyst is unable to reconstitute the tray on the same day. The initial heating stage is not needed, since it was performed during the first digestion. The program holds the tray at 50 °C after digestion to facilitate sample mixing after reconstituting. **This program should not be used as the initial digestion.**	1. Step to 380 °C 2. Hold for 60 min 3. Step to 50 °C 4. Hold for 450 min 5. Hold for 450 min 6. Hold for 450 min 7. End Program

5.5.14. Return the ALTQC, spiking, CCV, PQL, MDL SPK and calibration standard solutions to the refrigerator.

5.5.15. Check samples back into the receiving room.

5.5.16. Once the digestion is complete, carefully disconnect the data logger and place the tubes in a metal rack and allow the samples to cool under the hood.

Note: Before removing tubes from block, be prepared with heat resistant gloves in the event of a tube falling through the holding tray.

5.5.17. To obtain the temperature data from the data logger, repeat steps 5.5.12.1. and 5.5.12.2., above. Click on the probe entry that appears and click "Stop" in the Device tab.

Click "Download" to open the temperature report. Go to the Report tab and click on "Generate" button to generate data table. This will create a data table tab. Right click on the data table tab and select "Rename." Use the format "EPA 351.2_Tray number" to name the temperature file in the dialog box that opens. Use the "Save To" option to save the report on the Chemistry drive at\\floridadep\data\Labs\Lab3\Chem_Data\Inorganic\Nutrients\RunData\MetaData\TKN\DataLoggerFiles-TKN\. Print the report, which is to be included in the run folder. Ensure that temperature of the digestion stayed continuously at 380 C^{o} or above for at least 30 min. Mark the 30 min period on the temperature report. Initial and date the report.

Close the software.

5.5.18. Once the samples are cool enough to handle (typically after 15-30 minutes), use a pipette or bottle-top dispenser to add 25 mL of DI-Water.

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WARNING: The samples at this point consist of a concentrated sulfuric acid/salt mixture. The addition of DI water may generate much heat and thus poses a significant hazard. Be sure to point the tube away from your face and swirl gently to mix.

5.5.19. Parafilm the sample tubes individually, to prevent evaporation of the sample prior to analysis, and vortex until tube contents are fully mixed.

5.5.20. Check the bottom inside of the tubes for any precipitation. Salt samples are sometimes difficult to mix homogeneously because of the precipitation. If there is any difficulty getting the sample to mix well, place the sample, and all other tubes in the batch, into the water bath rack. Set the water bath to 60 °C. Once the bath reaches temperature, record the temperature in the log and place the rack with the tubes into the bath. Heat the tubes for approximately seven minutes. After heating, vortex the tubes and let them cool before pouring. Denote any batches that were heated on the analysis bench sheet.

Note: Tubes must be individually covered with parafilm during the heating process to prevent moisture loss through evaporation.

Note: Do not use the water bath cover when heating tubes, as this can cause condensation buildup that could drip down onto the tubes.

Digested solutions containing particulate matter should be filtered through a 0.45 μm filter prior to analysis and then redigested with dilution.

5.5.21. Review and complete the prep worksheet in the Prep Worksheet Manager module in LIMS:

5.5.21.1. Review the worksheet for correctness and add any necessary notes, changes, additions, etc.

5.5.21.2. Complete the worksheet by clicking the “Complete” button in the lower left corner of the worksheet – this changes the status of the samples from “W” to “V” in LIMS. Active or completed worksheets can be printed or exported in excel format for convenience when setting up the AQ2 analysis. The digestion worksheet itself is reviewed in LIMS directly and archived as part of the LIMS program.

5.5.22. The samples are now ready for analysis.

6. SAMPLE ANALYSIS/QUANTITATION/DATA ARCHIVAL

6.1. Refer to the DEP SOP NU-092 and DEP SOP NU-108 for analysis procedure, quantitation information, data archival, and method validation.

7. QUALITY CONTROL (SEE DEP SOPS NU-038 AND NU-043)

7.1. Samples with similar background matrices are grouped as a digestion batch and prepared together. A digestion batch is defined as a set of 10 samples or less from the same matrix group. The following are possible matrices that can be grouped together as

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a matrix group; each is prepared with one set of quality control samples. If a sample or group of samples is visibly different from the rest, they should be batched separately.

Matrix Group	Matrices grouped together as one batch
A	W-GROUND, W-SURF-FRH, and W-DRINKING
B	Conductivity > 5000 µmhos/cm
C	W-EFFLUENT, W-INFLUENT

Samples with the matrix WATER should be batched with Group A unless the conductivity is greater than 5000 µmhos/cm in which case they should be batched with Group B.

7.2. Digested MDL SPK - one per tray.

7.3. Digested PQCK2 - one per tray.

7.4. Digested standard reference material (ALTQC) - one per tray.

7.5. Digested LFB - one per batch.

7.6. Digested MBLK - one per batch.

7.7. Select samples for quality control – Randomly choose a sample to be used as the quality control sample for each batch. This sample may be changed if upon observation it seems to not be a representative of the rest of the samples, e.g. presence of a great deal of particulate or much different color. In this case you may treat this sample as a separate batch and continue with the sample preparation.

7.8. Sample matrix spike/s - Using the sample selected as QC sample (7.6.), prepare and digest a matrix spike or a set of duplicate spikes for each batch. If only one matrix spike is prepared the QC sample must be digested in duplicate.

7.9. 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 the following DEP SOP'S: NU-092, NU-108 and NU-043.

8. EQUIPMENT MAINTENANCE

8.1. Maintenance of the fume hoods and digestion blocks should be performed, at least, after every five uses. Maintenance must be documented in the block maintenance logbook. The maintenance for the fume hood is recorded in the logbook of the digestion block that is housed inside it.

8.2. The maintenance of both the fume hoods and digestion blocks include, but are not limited to:

8.2.1. The cleaning and wiping down of any dust and condensed sulfuric acid that has accumulated in the hoods.

8.2.2. The cleaning and wiping down of the digestion block to remove any corrosion or condensed sulfuric acid accumulation.

8.2.3. The replacement of bench paper that lines the bottom of the hood

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8.3. The maintenance of the temperature probes is documented in the temperature probe logbook under the appropriate section. Probe maintenance should be performed, at least, after every five uses. This includes, but is not limited to, inspection and cleaning. Any errors with probes reading correctly should be documented as well.

Note: Do not tilt the block and spray water into the wells to clean them out. This can cause water to drip into the block and may damage the block's insulation or heating element.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

9.1. Review the Laboratory Health and Safety Plan (accessible at https://floridadep.sharepoint.com/dear/SitePages/Lab%20Health%20and%20Safety.aspx).

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

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

9.4. Copper-containing waste –

The solution used to digest samples contains copper, which is a hazardous waste and toxic. Digestion solution being discarded and all sample waste that contains digestion solution (i.e., prior to or after digestion) should be collected in a hazardous waste container. These containers must have two labels: a hazardous waste label and a label to denote the start and fill date of the container.

Upon starting a waste container, denote the date in the 'Start Date' column of the Start/Fill label. Once a waste container is filled, denote the date under the 'Fill Date' column. A filled hazardous waste container can be stored for no longer than three working days in the prep lab. Further, no more than three full bottles of copper-containing waste should be stored in the prep lab.

The waste will be collected by trained personnel and disposed as acidic metals waste in the TKN Copper Waste drum in the hazardous waste room. After the empty waste container is returned, strike through the previous start and fill date. If the container was dumped prior to being full, strike through the previous start date.

9.5 Any concentrated acid used in the lab must be diluted down before it can be disposed of down the sink. For it to be considered tolerable for sink waste, it must be neutralized with sodium bicarbonate, or be diluted to 2% or by a dilution factor of 50.

9.6 The 1:1 HCl solution for cleaning is disposed of down the sink and should be flushed with copious amounts of tap water during the rinse procedures.

9.7 Before starting any heating sequence, the fumehood should be checked to ensure it is functioning properly and has sufficient air flow.

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10. 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.

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

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

10.3. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients Workgroups.

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

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

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

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

10.8. DEP SOP NU-046, Procedure for TKN/TP/LLTP Glassware Cleaning.

10.9. DEP SOP NU-049, Total Phosphorus (TP) Autoclave Sample Preparation.

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

10.11. DEP SOP NU-092, Determination of Total Kjeldahl Nitrogen in Water using the Seal AQ2 Discrete Analyzer

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

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

10.14. Quality Manual, Bureau of Laboratories, FDEP.

10.15. Laboratory Health and Safety Plan.

10.16. DEP SOP NU-108, Determination of Total Kjeldahl Nitrogen in Water using the SEAL Gas Diffusion Segmented Flow Analyzer

Appendix of Significant Changes

08/20/2010	Clarify instructions on procedure and requirements for the digestion of high conductivity samples (Sections 4, 5, and 7).
08/12/2014	Instructions are added for the use of the temperature logger systems and the preparation worksheet manager module within the LIMS program. (Section 3.10 and 5.4)

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05/06/2015	Use of pipettes to transfer sample aliquots into the digestion tubes. (Section 5.4.6. and 5.4.13)
07/26/2018	Section 8. **EQUIPMENT MAINTENANCE** – was added**.**
12/22/2020	In Section 3, the 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.
04/27/2023	Updated data logger software. Updated reference list to include items referenced in the SOP. Added preparation instructions for the standards and spiking solution to Section 4.3. Clarified and reorganized Section 5 to better reflect workflow. A list of block programs was added to Section 5.13. Updated heating procedure in Section 5.5.20. Clarified hazardous waste procedure for copper-containing waste in Section 9.4. Spike concentration raised from 1.2 mg N/L to 1.8 mg N/L. The concentration of the spiking solution was raised from 150 mg N/L to 225 mg N/L. The spike code was changed from O1 to O3. New spike concentration implemented for digestions starting on 10/21/2022.
9/17/2024	Updated file path links.
9/17/2025	Updated the expiration date for chemicals in section 4.1. Added a new bottletop dispenser in section 3.1 and the use of bottletop dispenser in adding the diluent in section 5.5.7. Updated the format and title for the prep worksheet in sections 5.5.3.2 and 5.5.17. Added instructions for disposing the 1:1 HCL cleaning solution and checking the fumehood in sections 9.6 and 9.7.
2/06/2026	MDL SPK solution was added to Section 4.3. Updated all mentions of tray standards to include the MDL SPK. Added the gas diffusion analysis SOP (DEP SOP-108) where necessary for analysis. Removed requirement to dilute salt samples in Section 5.

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