Nitrate Protocol

•Ellen Esch, May 2019, adapted from Doane, T. A., and W. R. Horwath. 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical Letters 36:2713-2722. And http://allison.bio.uci.edu/protocols/.

•Detection limit is <0.05 ppm. If you need lower, update the standard curve.

•If you need to extract from soils, you can do that, usually 15 g soil, 100 ml 2M KCl shaken for 60 min, settled overnight, filtered via Hart, S.C., Stark, J.M., Davidson, E.A. & Firestone, M.K. (1994) Nitrogen mineralization, immobilization, and nitrification. Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties (eds R.W. Weaver, J.S. Angle & P.S. Bottomley), pp. 985–1018. Soil Science Society of America, Madison, WI, USA.

•NOTE: "matrix" is DI water for rivers/streams. For resin bags it is likely 0.1 M HCl/2.0 M NaCl, soil extracts KCl. (salinity matters)

Make solutions:

1 M HCl

- Add 500 ml ultrapure water to 1 liter volumetric flask
- Add 84 ml concentrated HCl to flask and swirl to mix, then bring to 1 liter with ultrapure water
- Store, but if needed neutralize with sodium bicarbonate and dispose down drain

Saturated vanadium chloride solution

- Add 0.35 g vanadium (III) chloride to 50 ml of 1 M HCl; filter if necessary
- CAUTION: the vanadium chloride powder is very reactive with air! Work quickly.
- Toxic!!! Very bad for eyes!! Wear PPE, don't inhale/eat. Will not be disposing. Not regulated but should probably be labeled as HAZARDOUS WASTE if needed.

2% sulfanilamide solution

- 0.2 g sulfanilamide
- 10 ml of 1 M HCl
- Not regulated but should probably be labeled as HAZARDOUS WASTE.

0.2% NED solution

- 0.02 g N-(1-naphthyl)-ethylenediamine dihydrochloride
- 10 ml ultrapure water
- Not regulated but should probably be labeled as HAZARDOUS WASTE.

Reagent solution

1. 50 ml saturated vanadium chloride solution
2. 3.3 ml 2 % sulfanilamide solution
3. 3.3 ml 0.2 % NED solution
4. 400 ml nanopure water
5. Purge 17 ml aliquots with nitrogen or helium and store for up to 3-12 months frozen

- Will store, but if need to dispose, no items are regulated, but should probably be labeled as HAZARDOUS WASTE.

Stock nitrate solution (100 ppm)

- Add 0.361 g potassium nitrate KNO_3 to a 500 mL volumetric with nanopure
  - (0.1 g N / 1 L) * (101.1032 g KNO_3 / 14.007 g N) * (0.5 L) = 0.361 g KNO_3
  - (desired ppm) * (percent N) * (desired volume) = mg KNO_3 to add to 500 mL
  - 100 ppm N = (100 µg N / 1 mL) = (0.1 g N / 1 L)
- High concentrations are toxic to fish, so should label as HAZARDOUS WASTE.

Determine how many plates you need, and create a set-up:

1. If running triplicates = each plate will have a standard curve (6 levels * 3 reps = 18 wells), so you can fit in 26 samples (78 / 3).
2. If running quadruplicates = each plate will have a standard curve (6 levels * 4 reps = 24 wells), so you can fit in 18 samples (72 / 4).

Make standard curve:

Generally low curve for unfertilized. From fertilized areas, **sample should be diluted in matrix and run using the high protocol.

1. Dilute the 100 ppm stock solution to either:
   •(low) 1 ppm, add 1 ml of 100 ppm stock solution to 100 ml volumetric flask
   •(high) 10 ppm; add 10 ml of 100 ppm stock solution to 100 ml volumetric flask
2. Create the following standard curves in 1.5 ml centrifuge tubes.

Low

[Std]	µl 1 ppm	µl matrix
0 ppm	0	1000
0.05 ppm	50	950
0.10 ppm	100	900
0.20 ppm	200	800
0.50 ppm	500	500
1.00 ppm	1000	0

High

[Std]	µl 10 ppm	µl matrix
0 ppm	0	1000
0.5 ppm	50	950
1.0 ppm	100	900
2.0 ppm	200	800
5.0 ppm	500	500
10.0 ppm	1000	0

Run analysis:

NOTE: run samples in triplicate (or quadruplicate!!) and add the standard curve to each plate. Add the samples/standards to the wells first, and then use the multichannel pipette to add the reagent. Make sure to label plates and create a diagram for your sample layout. If samples do not retain a plate to bright pink color, the samples are too concentrated or the reaction is gone too far, dilute them!

For low concentrations (0-5 ppm), add the following to each well:

1. 100 µl sample
2. 100 µl reagent solution (use multichannel pipet)

For high concentrations (1-10 ppm):

1. 10 µl sample
2. 160 µl reagent solution (use multichannel pipet)

Tap corner of plate to mix well, cover with foil and incubate for 5 hours. Read plate at 540 nm.

Reporting results:

"Concentration of nitrate was determined colorimetrically using an Epoch microplate reader (BioTek, VT, USA) with the vanadium reduction method (Doane and Horwath 2003). Samples were run in triplicate (or quadruplicate), and outliers were determined by xyz."

Waste disposal:

Most reagents will get stored for future use. Look in hood for labeled hazardous waste containers (might need to create a new one). Package plates in plastic bag (in hood). If things are getting full (they cannot be >75% full!!!), please fill out this form from EHS and email it to ehs@uoguelph.ca to request pickup.