Total Nitrogen analysis

Total nitrogen (or total soluble, if the sample is filtered) can be assayed by oxidation with alkaline persulphate to nitrate, followed by analysis using the hydrazine reduction technique [modified from APHA, 1995].

Reagents:
Digestion:

Digestion reagent:

Potassium persulphate $K_2S_2O_8$ (AR)	20.1g
Sodium Hydroxide NaOH (AR)	3.0g
Deionized water to	1000ml

Make appropriate volume just prior to use

Borate buffer:

Boric acid H_3BO_3 (AR)	61.8g
Sodium Hydroxide NaOH (AR)	8.0g
Deionized water to	1000ml

Digestion check Standards:

Glutamic acid stock solution:
dry glutamic acid at 105°C for 24h
Dissolve 1.051g in deionized H_2O, dilute to 1000ml 1.00ml=100µg N
preserve with 2ml $CHCl_3$

Glutamic acid intermediate solution: Dilute stock 1 in 10 to give 10µg per ml
for digestion check standard, dilute 29 ml of stock to 100ml = 2.9 $\mu g.ml^{-1}$

[Yeast or Yeast Extract:
Suspend/dissolve 102mg in 100ml H_2O: total N should be $2mg.l^{-1}$. [total P in this standard should be $3.97mg.l^{-1}$.] This has been tested as a digestion check standard, but the results are equivocal]

Nitrate assay:

NaOH reagent: 250ml *caution: caustic*

NaOH 25g, add to 237.5 ml deionized H_2O, store in air tight plastic bottle at room temp. for 1 month.

Reducing reagent: *caution: poisonous, highly reactive*

A:	Hydrazine sulphate	2.7g in 100ml deionized H_2O (heat to dissolve)	*caution: poisonous*
B:	Copper sulphate ($CuSO_4 \cdot 5H_2O$)	0.25g in 100ml deionized H_2O	*caution: harmful*
C:	Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	5.3 g in 100ml deionized H_2O	*caution: harmful*

Store all solutions at room temp: stable indefinitely until mixed.

On day of use mix: 27.4 ml H_2O; 10ml A; 1.5ml B; 1.1ml C.
(do not use if more than 1 day old)

Colour Reagent: add in the following order:

deionized H_2O	90ml
Orthophosphoric acid	50ml
Sulphanilamide	5g
N-1-naphthylethylenediamine dihydrochloride (NEDD)	0.25g
dissolve and add (use for rinsing reagents in, etc.) deionized H_2O	57.5 ml

store refrigerated in a dark bottle: renew monthly

Standard Nitrate solution:

KNO_3 (anhydrous) 7.221g
Dissolve in deionized H_2O to 1000ml (volumetric flask) @ 20°C: add 2 ml Chloroform as preservative: keep for no longer than 1 week.
This stock is 1000mg/l **NO_3 - N**. Dilute to 5000µg.l^{-1} for working strength: this solution should not be stored. make up standards for analysis up to 3000µg.l^{-1}.

Method:

1 add 20 ml of standard solutions or sample (suitably diluted) to pre-weighed 50-100 ml conical flasks (cleaned first by autoclaving with digestion reagent: thereafter by acid-washing)

2 add 10 ml of digestion solution, mix. Cover flasks with small glass beakers.

3 Place in autoclave: heat for 30 minutes at 121 °C.

4 Allow to cool to room temperature, check volume (by weighing) and make up to 30 ml if necessary: add 2 ml borate buffer, mix.

5 Distribute 5 ml aliquots from each flask to 3 acid-washed glass test tubes .

6 Add 725µl NaOH reagent, mix (vortex).

7 Add 420µl of freshly made reducing agent and mix.

8 Wait 4-5 minutes (or more)

9 Add 725µl Colour reagent, mix.

10 Leave for 30 min for full colour development, mix and read at 535nm.
Absorbance of a 1000µg/l standard in a 1 cm cell should be approx. 0.5-0.7.

References

APHA. 1995. Standard Methods for the Examination of Water and Wastewater, 19th edition. American Public Health Association.

D'Elia, CF and Steudler, PA. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnology and Oceanography. 22. 760-764.

Dilutions for standard curve: volumes to make 20ml for digestion reaction

Concentration µg/l	5 mg/l stock	Water
0	-	20ml
500	2ml	18
1000	4	16
1500	6	14
2000	8	12
2500	10	10
3000	12	8

Total Phosphorus Analysis:

by acid persulphate digestion followed by ascorbic acid procedure.

For Total Soluble P (TSP), filter the water sample before analysis, otherwise the procedure gives Total P (TP)

Reagents:

30% H_2SO_4	30 ml conc H_2SO_4 added to 50ml deionised water, made up to 100 ml when cool. *Caution highly corrosive, great heat emitted during dilution: dilute into beaker in a basin of cold water, in fume hood.*
8% potassium persulphate:	8g potassium persulphate dissolved in 100 ml deionised H_2O, stirring on a hotplate to dissolve. Prepare just before use and dispense while still warm.

Procedure:

1. Add 5 ml of sample, standards, and blank to rewashed Hach COD tubes, in triplicate.
2. Add 0.1 ml 30% H_2SO_4 and 0.5 ml fresh potassium persulphate solution.
3. Put screw caps on tubes and tighten
4. Place in autoclave and autoclave for 30 min at 15 psi (121°C)
5. Allow to cool
6. Add 1 ml of freshly prepared mixed reagent (see Ascorbic acid method) and vortex to mix.
7. Leave 20-30 min, read absorbance at 882nm in a 1cm flowcell zeroed against a deionised water blank