# ASSIMILABLE AMINO NITROGEN PROCEDURE

**RAPID METHOD 1998**

## Equipment

- Spectrophotometer, UV at 335nm, zero on DI water
- Cuvettes, 10mm
- Pipettor, 50µL
- Adjustable micro-pipettor with disposable syringes
- 50 mL centrifuge tubes
- Centrifuge

## Reagents

- NAC Buffer (Reagent buffer-No OPA)
- OPA Solution, 5%
- 10mM Isoleucine Standard (10mM Ile)
- DI Water

## Procedure

Mix reagents as needed:

**NAC Buffer**, pour premeasured crystalline NAC into premeasured borate solution. Use within 2 days of mixing. Refrigerate for stability and use at room temperature.

**5% OPA**, add the contents of ethanol vial (5 mL) to the premeasured OPA. Use within 1 day.

**STANDARD CURVE**

1. Add 3 mL NAC buffer into cuvettes. Add 10mM Ile standard and water as follows:

| Cuvette | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 10mM Ile | 0 | 10µL | 30µL | 50µL |
| DI Water | 50µL | 40µL | 20µL | 0 |

2. Add 50µL 5% OPA solution to each cuvette.
3. Read Absorbance values and record in log book.



**Revision:** August 2019

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## Samples

Setting up calculations in EXCEL is most expedient.
Note: To convert to ppm Nitrogen, the multiplication factor is 14.

1. Key in (on HP scientific calculator)

| | | |
|---|---|---|
| $f$ | GSB | (clears statistical register) |
| Concentration value 1( y ), Enter | | |
| Absorbance value 1 ( x ) Σ+ | | |
| Concentration value 2, Enter | | |
| Absorbance value 2 Σ+ | | |
| Etc…… | | (Enter all the concentration (y) and absorbance (x) values) |
| $f$□Σ□ | | (Calculates the linear regression equation and displays the y-intercept) |
| X ⇔ Y | | (Displays the slope) |
| $f$•,□then X ⇔ Y | | (Displays r value) |
| Sample<br>Absorbance value $f$• | | (Displays concentration value)<br>(multiply by 14 to convert to ppm Nitrogen) |

## Disposal

Dispose in sink.



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