

## Microplate Nutrient Analysis

(Modified from Kathleen Treseder's protocol)

### Ammonium Analysis

Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39:971-974

#### Materials

- Matrix (solution used to extract samples: usually 2.0M KCl or 0.5M K<sub>2</sub>SO<sub>4</sub>)
- Sodium salicylate solution (in refrigerator)
- 2% bleach in 1.5M sodium hydroxide (make fresh every day: see recipes)
- Stock Ammonium Solution (100 ppm, on lab bench)
- Sterile 96-well microplate: Fisher #08-772-7
- Microplate lids: Fisher # 08-772-2B
- Micropipettes and tips
- Multichannel pipette
- Pipetting Basin: Fisher # 13-681-100
- 1.5 ml microfuge tubes: Fisher # 05-408-129

#### Notes on "high" vs "low" protocols:

Samples expected to have low nitrogen (N) content (0-1 ppm) should be run using the "low" protocol. Samples expected to have high N (e.g., from fertilized treatments) should be run using the "high" protocol. If unsure, run samples using the "high" protocol.

#### Procedure

##### 1) Make a standard ladder.

Dilute the 100 ppm stock ammonium solution into a microfuge tube:

*High concentrations:* 150 µl stock + 1350 µl matrix per tube, for a total of 1.5ml of 10 ppm ammonium solution.

*Low concentrations:* 15 µl stock + 1485 µl matrix per tube, for a total of 1.5ml of 1 ppm ammonium solution.

Using the diluted stock solution and the matrix solution, create the following standard curves in 6 more microfuge tubes.

Standard ladder : HIGH		
Concen.	µl 10 ppm	µl matrix
0.0 ppm	0	1000
0.5	50	950
1.0	100	900
2.0	200	800
5.0	500	500
10.0	1000	0

Standard ladder : LOW		
Concen.	µl 1 ppm	µl matrix
0.00 ppm	0	1000
0.05	50	950
0.10	100	900
0.20	200	800
0.50	500	500
1.00	1000	0

**2) Load the plate.**

Add the following to each well of a 96-well microplate (load ALL the samples first before adding the reagents):

High concentrations:

- 20 µl sample (ladder solutions are added the same as samples)
- 90 µl sodium salicylate solution (add using multichannel pipette)
- 90 µl bleach solution (add using multichannel pipette)

Low concentrations:

- 40 µl sample (ladder solutions are added the same as samples)
- 80 µl salicylate solution (add using multichannel pipette)
- 80 µl bleach solution (add using multichannel pipette)

Load the standard ladder first, into column 1. Because there are 8 wells in each column and only 6 standards, load the lowest (0.0 ppm) and highest (10.0 ppm) twice. (From well A1 – H1, it will be: 0.0, 0.0, 0.5, 1.0, 2.0, 5.0, 10.0, 10.0) Run a standard ladder with EVERY plate.

After loading the ladder and all the samples, use the multichannel pipette to add the sodium salicylate and bleach/NaOH solutions. Pipette up and down in each well to mix.

**3) Incubate for 50 min – 1 hr and read plate at 650 nm.** Detection limit <0.05 ppm.

\*Remember to record which samples go into each well. Sample data sheet:

Date: \_\_\_\_\_ Name of preparer: \_\_\_\_\_ Nutrient: \_\_\_\_\_  
 Time finished preparing: \_\_\_\_\_ Time to run on plate reader: \_\_\_\_\_  
**Plate description / number:** \_\_\_\_\_

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

\*The samples will turn a blue green color; however, if the concentration of ammonium is too high, the reaction will go too far and the sample will turn yellow. If this happens the sample should be diluted and ran again. (Common dilution for sample: 100 µl sample + 900 µl matrix)

\*Remember to run a control (a sample that did not need to be diluted) with your diluted samples, to double check that your dilutions worked.

**Nitrate Analysis**

Doane, T. A., and W. R. Horwath. 2003. Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters* 36:2713-2722.

Materials Needed:

- Matrix (solution used to extract samples: usually 2.0M KCl or 0.5M K<sub>2</sub>SO<sub>4</sub>)
- Vanadium solution
- Stock nitrate solution
- Sterile 96-well microplate: Fisher #08-772-7
- Microplate lids: Fisher # 08-772-2B
- Micropipettes and tips
- Multichannel pipette
- Pipetting Basin: Fisher # 13-681-100
- 1.5 ml microfuge tubes: Fisher # 05-408-129

Notes on “high” vs “low” protocols:

Samples expected to have low nitrogen content (0-1 ppm) should be run using the “low” protocol. Samples from fertilized treatments should be run using the “high” protocol. If unsure, run samples using the “high” protocol.

Procedure**1) Make a standard ladder.**

Dilute the 100 ppm stock nitrate solution into a microfuge tube:

*High concentrations:* 150 µl stock + 1350 µl matrix per tube, for a total of 1.5ml of 10 ppm nitrate solution.

*Low concentrations:* 15 µl stock + 1485 µl matrix per tube, for a total of 1.5ml of 1 ppm nitrate solution.

Using the diluted stock solution and the matrix solution, create the following standard curves in 6 more microfuge tubes.

Standard ladder : HIGH		
Concen.	µl 10 ppm	µl matrix
0.0 ppm	0	1000
0.5	50	950
1.0	100	900
2.0	200	800
5.0	500	500
10.0	1000	0

Standard ladder : LOW		
Concen.	µl 1 ppm	µl matrix
0.00 ppm	0	1000
0.05	50	950
0.10	100	900
0.20	200	800
0.50	500	500
1.00	1000	0

**2) Load the plate.**

Add the following to each well of a 96-well microplate (load ALL the samples first before adding the reagents):

High concentrations:

10 µl sample

160 µl Vanadium solution

Low concentrations:  
 100 µl sample  
 100 µl Vanadium solution

Load the standard ladder first, into column 1. Because there are 8 wells in each column and only 6 standards, load the lowest (0.0 ppm) and highest (10.0 ppm) twice. (From well A1 – H1, it will be: 0.0, 0.0, 0.5, 1.0, 2.0, 5.0, 10.0, 10.0) Run a standard ladder with EVERY plate.

After loading the ladder and all the samples, use the multichannel pipette to add the vanadium solution. Gently tap the corner of the plate to mix.

**Incubate 5 hrs or overnight and read plate at 540 nm.** Detection limit <0.05 ppm.

\*Remember to record which samples go into each well. Sample data sheet:

Date: \_\_\_\_\_ Name of preparer: \_\_\_\_\_ Nutrient: \_\_\_\_\_  
 Time finished preparing: \_\_\_\_\_ Time to run on plate reader: \_\_\_\_\_  
**Plate description / number:** \_\_\_\_\_

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Converting the absorbance to concentration:

The plate reader provides an absorbance value for the sample, which needs to be converted to a concentration (ppm). To convert:

- Create an XY scatter plot using the standard curve data: ppm vs absorbance
- Fit a linear regression to the data and calculate the equation of the line and the R<sup>2</sup> value of the data. The R<sup>2</sup> should be as high as possible.
- Use the equation of the regression line to convert the absorbance data of each sample to ppm.
- Adjust your sample concentrations with the control (sample ppm – control ppm) to calculate the final concentration for each sample.
- To determine the amount (mg) of nitrate or ammonium, refer back to the inorganic soil N extraction protocol.