

Microplate measurement of ammonium

DISCLAIMER

Before using this or any other analytical method it is imperative that you check that it works with your samples. The bare minimum is to test accuracy and precision.

- Test accuracy by creating a standard curve by serial dilution of a sample and/or via spike and recovery tests. Both tests will show if the analysis is affected by the sample matrix.
- Test precision by repeated analysis of the same sample. It's best to do separate precision tests for the analytical method (replicate analyses of the same extract) and for the entire extraction and analysis procedure (extract the same sample several times and carry each extract through the analysis procedure). These tests will show you where poor precision is creeping into your analysis.

Remember that your results are qualitative if you rely on a standard curve with a purified analyte.

Method adapted from:

Baethgen WE, Alley MM (1989) A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digest. Commun Soil Sci Plant Anal 20 (9&10) 961-969

Prepare stock solutions

Make sodium salicylate cocktail

- To a 100 mL flask add 60 mL of water
- Add 6.8 g sodium salicylate
- Add 5 g of (tri)sodium citrate
- Add 5 g of sodium tartrate
- Add 0.025 g of sodium nitroprusside (**caution: toxic!**)
- Dissolve and make up to 100 mL with H₂O

Make NaOH solution

- Make solution of 60 g L⁻¹ NaOH (**caution: corrosive!**) by dissolving 6g of NaOH in 100 mL H₂O

Make hypochlorite/NaOH solution

- This solution is unstable, so only make enough solution for a day (50 mL is enough for ~ 4 plates)
- To make 50 mL, dilute 1 mL of 10% hypochlorite (**caution: corrosive!**) to 50 mL with 60 gL⁻¹ NaOH

Make 10 mM ammonium-N stock solution

- In a 100 mL flask dissolve 0.066 g of (NH₄)₂SO₄ (this gives a 5mM solution of ammonium sulphate which equates to 10 mM of ammonium)
- Store in dark at 4°C

Make 200 μM ammonium N solution

- Pipette 2mL of 10 mM stock solution into 100 mL vol flask
- Make up to 100.0 mL using extractant (e.g. KCl) or sample matrix

Make ammonium std curve solutions

- Table below is for 10 mL final volume. Scale appropriately for other volumes

Conc (μM)	mL of 200 μM	mL of matrix
0	0.0	10.0
10	0.5	9.5
20	1.0	9.0
40	2.0	8.0
80	4.0	6.0
120	6.0	4.0
160	8.0	2.0
200	10.0	0.0

Analysis procedure

- Pipette 40 μL of sample or standard (0-200 μM) into 96-well plate
- Add 80 μL of salicylate cocktail
- Add 80 μL of hypochlorite. (pipette up and down to mix well)
- Let rest for 45 mins at room temperature
- Read absorbance at 650 nm

Notes

- Samples will turn emerald green
- If concentration of ammonium is too high dilute samples, or else change relative amounts of sample and reagents (e.g. 20 μL of sample, 100 μL of salicylate, 100 μL of hypochlorite)

