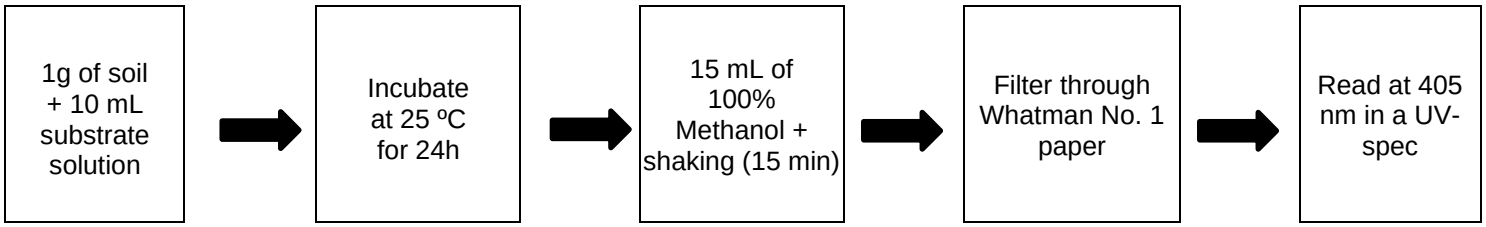
SOP: **Soil Deaminase Assay**

Overview:

This standard operating procedure (SOP) describes a protocol for measuring the potential deaminase activity rates in soil. Originally reported by Killham and Rashid (1986) and modified by Allison (1990), this method differs from others that use natural substrate and quantify change in extractable ammonium. Key instruments are a laboratory water bath (25˚C), a macro-centrifuge machine, filter paper, a dispensette (15 mL) or pipette (10mL), and an ultra-violet spectrophotometer. A key safety consideration is the use of 100% methanol, which must be handled under a fume hood. Soils that are ground to pass a 2 mm sieve are typically used.



Safety:

All standard safety protocols and online safety training via UIUC Division of Research Safety (DRS) are required.
Personal protection (PPE) for this procedure include:
Body Protection: Laboratory coat
Hand Protection: Nitrile gloves
Particularly hazardous substances: 100% Methanol

- ✓ Keep away from heat/ sparks/ open flames/ hot surfaces. No smoking.
- ✓ Keep container tightly closed.
- ✓ Wash skin thoroughly after handling.
- ✓ Do not eat, drink or smoke when using this product.
- ✓ Use only outdoors or in a well-ventilated area.
- ✓ Wear protective gloves/ eye protection/ face protection.
- ✓ IF SWALLOWED: Immediately call a POISON CENTER/ doctor. Rinse mouth.
- ✓ IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower.
- ✓ IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor.
- ✓ IF exposed: Call a POISON CENTER or doctor/ physician.
- ✓ Take off contaminated clothing and wash before reuse.
- ✓ In case of fire: Use dry sand, dry chemical, or alcohol-resistant foam to extinguish.
- ✓ Store in a well-ventilated place. Keep cool. Store locked up.
- ✓ Dispose of contents/ container to an approved waste disposal plant.

Instrumentation & Consumables:

Sample and substrate preparation

- Analytical balance (up to three decimal places)
- 50 mL disposable centrifuge tubes (Falcon tubes)
- Styrofoam centrifuge tube racks
- 4-nitro-o-phenylenediamine (MW: 153.14)
    - Also called 1,2-diamino-4-nitrobenzene (1,2-DANB)
- 18.2MΩ-cm water

Reaction

- 10 mL pipette and tips
- Laboratory water bath capable of maintaining 25ºC for 24 hours
- Plastic wrap
- Metal/plastic tray

Extraction / Purification (filtration)

- Fume hood
- Methanol (100%)
- Dispensette (15 mL) or pipettes and tips (10 mL, 5 mL)
- Laboratory horizontal shaker (reciprocating shaker, 100-150 rev min^{-1})
- Macro-centrifuge
- Whatman No. 1 filter paper

Spectrophotometry

- Ultra-violet spectrophotometer
- 96-well microplates
- 200 µL pipette and tips

Detailed Procedure:

I. Substrate preparation (4-nitro-o-phenylenediamine, 0.392 mM, 500 mL)

1. Dissolve 0.030 g of 4-nitro-o-phenylenediamine (red in color) in 500 mL of 18.2MΩ-cm water using a magnetic stirrer and stir bar for ~ 30 min.

II. Sample preparation

1. In 50 mL centrifuge tubes, add 1 ±.01 g (air-dry or oven-dried equivalent) of soil.
2. To control for abiotic degradation of the substrate, include at least 1 empty (e.g. soil-free) tube.

III. Standard preparation (for standard curve)

1. In 50 mL centrifuge tubes, prepare a series of standard solutions by adding 4-nitro-o-phenylenediamine substrate solution (0.392 mM), 18.2MΩ-cm water, and methanol (100%), as follows:

Standard solutions preparation						
Substrate solution (mL)	10	5	2.5	1.25	0.625	0
18.2MΩ-cm water (mL)	0	5	7.5	8.75	9.375	10
100% methanol (mL)	30	30	30	30	30	30
Total (mL)	40	40	40	40	40	40

***Note that these standards are only stable for 2 hours because the methanol evaporates*.**

IV. Reaction

1. To each tube (samples and control), add 10mL of substrate solution (4-nitro-o-phenylenediamine, 0.392 mM).
2. Swirl the centrifuge tubes for 1-2 min.
3. Place plastic wrap on top of the tubes to avoid evaporation during incubation.
4. Incubate the tubes at 25 °C for 20-24 hours (standardized by project) using a water bath.
    i. To keep the tubes inside the water bath, place weight – e.g., tray + flasks/bottles filled with water – on top of the tubes.

V. Extractions

1. First extraction and filtration
    1.1. Remove samples from the water bath. Under a fume hood, add 15 mL of 100% methanol to each sample.
    1.2. Place the samples in a horizontal shaker at low speed (100-150 rev min^{-1}) for 15 minutes.
    1.3. Centrifuge the tubes at 4000 rev min^{-1} for 5 minutes.
    1.4. Filter (all) resulting slurry through a Whatman No. 1 filter paper until the remnant is moist soil only.
    Note: The soil-free control does not need to be filtered
2. Second extraction and filtration
    2.1. Under a fume hood, add an additional 15 mL of 100% methanol to each moist soil sample, shake for another 15 min, centrifuge, and filter (i.e. repeat steps 1.1., 1.2., 1.3., and 1.4.)
        2.1.1. For the controls, add an additional 15 mL of 100% methanol to the original, unfiltered solution.

3. Extraction Combination
    3.1. Combine the first and second extraction of each sample into a new tube (final volume= 40 mL).
    3.2. Cap and invert the tubes 2-3 times to ensure solution is well mixed prior to colorimetry.

VI. Colorimetry

1. Transfer 200 µL of the extractions, controls, and standards into a 96-well plate.
2. Read absorbance at 405 nm using an ultra-violet spectrophotometer.

VII. Calculations

Measurement of deaminase activity is usually expressed in units of µmol substrate deaminated g^{-1} soil h^{-1}. To calculate, see formula below:

F = final substrate concentration (mM), converted using standard curve
C = correction for abiotic degradation of 1,2-DANB (mM), converted using standard curve
W = soil mass (g)
T = time incubated (h)

0.09795 = Initial substrate concentration (mM)
1000 = converts millimolar concentration to micromolar
0.04 = final volume (L)

$$\mu mol\ substrate\ deaminated\ g^{-1} soil\ h^{-1} = \frac{[(0.09795 - \mathrm{F}) - (0.09795 - \mathrm{C})] * 1000 * 0.04}{W * T}$$

Example calculation:

Standard curve: y = 0.1986x - 0.0092

Sample absorbance: 0.138
Sample converted to mM: 0.1986 * 0.138 - 0.0092 = 0.0182 mM 1,2-DANB

Control absorbance: 0.527
Control converted to mM: 0.1986 * 0.527 - 0.0092 = 0.0955 mM 1,2-DANB

$$\frac{[(0.09795 - 0.0182) - (0.09795 - 0.0955)] * 1000 * 0.04}{0.99 * 24}$$

$= 0.130\ \mu mol\ 1,2\text{-}DANB\ g^{-1}\ soil\ h^{-1}$

VIII. Clean up

1. Dispensette should be cleaned **immediately** after use to prevent damage from methanol. Return to maximum volume, then pump deionized water through dispensette 5-10x. Empty and allow to dry before storing.
2. Centrifuge: remove Falcon tube holders and wipe away any liquid at the bottom.
3. Dispose of substrate solution + 100% methanol into a closed waste container with labels.
4. Falcon tubes and filters may be thrown away in regular trash bins.

References:

Allison 1990. Deaminase activity in arable soils. *Plant and Soil 126*: 109-113. https://doi.org/10.1007/BF00041375

Killham and Rashid 1986. Assay of activity of a soil deaminase. *Plant and Soil 92*: 15-21. https://doi.org/10.1007/BF02372261

Suggested reading:

Hopkins, D.W., O'Donnell, A.G. and R.S. Shiel. 1988. The effect of fertilisation on soil nitrifier activity in experimental grassland plots. *Biol. Fertil. Soils 5*: 344-349. https://doi.org/10.1007/BF00262144

Citation:

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