Solid-Nitrate Reductase(S-NR) Activity Assay Kit

Product Code: 67106

Soil Nitrate Reductase (S-NR) Activity Assay Kit - Spectrophotometric Method

Product Information

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Soil nitrate reductase (S-NR) catalyzes the reduction of nitrate to nitrite in soil and is a key enzyme in soil nitrate nitrogen reduction. Studying S-NR activity is important for rational fertilization and reducing nitrogen loss.

S-NR catalyzes the reaction: NO_3^- + NADH + H^+ -> NO_2^- + NAD^+ + H_2O.

Under acidic conditions, the nitrite produced reacts with p-aminobenzenesulfonic acid and alpha-naphthylamine to quantitatively form a red azo compound with a maximum absorption peak at 540 nm.

Package Contents

Catalog Number	Component	Quantity
67106.1	Reagent I	1 bottle
67106.2	Reagent II	1 bottle
67106.3	Reagent III	1 bottle
67106.4	Reagent IV	1 bottle
67106.5	Reagent V	1 bottle
67106.m	Manual	1 copy

Quality and Safety Information

Component	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Reagent IV	--	--
Reagent V	--	--

Transport and Storage

Transport	Transport with ice packs.
Storage	Store Reagent III and Reagent IV at 2-8 degrees C protected from light. Store the remaining reagents at -20 degrees C. Shelf life: 180 days.

Instructions for Use

1. Sample Processing

Air-dry fresh soil samples naturally or dry them in a 37 degrees C oven, then pass the samples through a 30-50 mesh sieve.

2. Reagent Preparation

- **Reagent III:** If crystals precipitate, dissolve them in a 60-90 degrees C water bath before use.
- **Reagent V:** Standard stock solution, 1 mL. Store at -20 degrees C.
- **0.1 micromol/mL standard solution:** Dilute Reagent V 100 times when needed. Take 0.1 mL and add distilled water to a final volume of 10 mL.

3. Assay Procedure

Add the following reagents to capped centrifuge tubes.

Component	Assay Tube	Control Tube	Standard Tube	Blank Tube
Air-dried soil sample (g)	0.1	0.1		
0.1 micromol/mL standard solution (microliter)	100			
Distilled water (microliter)	375	475		
Reagent I (microliter)	375			
Reagent II (microliter)	125	125	125	125

1. Mix, cover with lids, and incubate in a 37 degrees C water bath for 24 h.
2. Centrifuge at 8000 g, 25 degrees C for 10 min, and collect the supernatant.
3. Add the following reagents to new tubes as shown below.

Component	Assay Tube	Control Tube	Standard Tube	Blank Tube
Supernatant (microliter)	400	400	400	400
Reagent III (microliter)	250	250	250	250
Reagent IV (microliter)	250	250	250	250

1. Mix and allow color development at 25 degrees C for 20 min.
2. Centrifuge at 4000 g, 25 degrees C for 10 min.
3. Zero the instrument with distilled water.
4. Read the absorbance of each tube at 540 nm.

The standard tube and blank tube only need to be measured once. Set one control tube for each assay tube.

> Before formal testing, perform 2 sample pre-assays. Due to the special nature of a small number of soils, assay specificity may vary.

4. Activity Calculation

Unit definition: The amount of g soil sample that produces 1 micromol NO_2^- per day is one S-NR activity unit.

S-NR (micromol/d/g soil sample) = $C_{standard}$ x ($A_{assay\ tube}$ - $A_{control\ tube}$) / ($A_{standard\ tube}$ - $A_{blank\ tube}$) x $V_{total\ reaction}$ / W / T

S-NR (micromol/d/g soil sample) = 0.5 x ($A_{assay\ tube}$ - $A_{control\ tube}$) / ($A_{standard\ tube}$ - $A_{blank\ tube}$)

- $C_{standard\ tube}$: standard tube concentration, 0.1 micromol/mL
- $V_{total\ reaction}$: total volume of the reaction system, 0.5 mL
- T: reaction time, 1 d
- W: sample mass, 0.1 g

Precautions

1. Before formal measurement, select 2-3 samples with large expected differences for prediction.
2. Required instruments and supplies to be prepared by the user: visible spectrophotometer, water bath, benchtop centrifuge, adjustable pipette, 1 mL glass cuvette, and distilled water.
3. The 50T kit measures 24 samples.

Visual Reference