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

Product Code: 67112

Soil Nitrate Reductase (S-NR) Activity Assay Kit - Microplate 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. Measuring S-NR activity is useful for studies related to rational fertilization and reducing nitrogen loss.

S-NR catalyzes the following reaction:

$NO_3^- + NADH + H^+ \rightarrow NO_2^- + NAD^+ + H_2O$

Under acidic conditions, the generated nitrite reacts with p-aminobenzenesulfonic acid and α-naphthylamine to quantitatively form a red azo compound. This compound has a maximum absorbance peak at 540 nm.

Actual readings may vary depending on the testing instrument and conditions.

Package Contents

Size	Item Code	Component	Quantity
100T	67112.1	Reagent I	1 bottle
100T	67112.2	Reagent II	1 bottle
100T	67112.3	Reagent III	1 bottle
100T	67112.4	Reagent IV	1 bottle
100T	67112.5	Reagent V	1 bottle
100T	67112.m	Manual	1 copy

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Reagent IV	--	--
Reagent V	--	--

Shipping and Storage

Shipping	Shipped with ice packs.
Storage	Store Reagent III and Reagent IV at 2-8°C, protected from light. Store all other reagents at -20°C.
Shelf Life	180 days.

Instructions for Use

1. Sample Processing

Naturally air-dry fresh soil samples or dry them in a 37°C oven. Pass the dried samples through a 30-50 mesh sieve.

2. Reagent Preparation

- **Reagent III:** If crystals precipitate, dissolve before use in a 60°C-90°C water bath.
- **Reagent V:** Standard stock solution, 1 mL. Store at -20°C.
- **0.1 μmol/mL standard solution:** Dilute Reagent V 100 times before use. Add 0.1 mL of Reagent V and dilute with 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)	0.06	0.06		
0.1 μmol/mL standard solution (μL)	60			
Distilled water (μL)	225	285		
Reagent I (μL)	225	225		
Reagent II (μL)	75	75	75	75

1. Mix well, cover the tubes, and incubate in a 37°C water bath for 24 h.
2. Centrifuge at 8000 × g, 25°C for 10 min, and collect the supernatant.
3. Add the following reagents to new tubes:

Component	Assay Tube	Control Tube	Standard Tube	Blank Tube
Supernatant (μL)	130	130	130	130
Reagent III (μL)	85	85	85	85
Reagent IV (μL)	85	85	85	85

4. Mix well and allow color development at 25°C for 20 min.
5. Centrifuge at 4000 × g, 25°C for 10 min.
6. Transfer 200 μL of supernatant to a 96-well plate.
7. Zero the instrument with distilled water and read the absorbance of each tube at 540 nm.

> The standard tube and blank tube only need to be measured once. Prepare one control tube for each assay tube.
>
> Before formal measurement, perform a preliminary test using 2 samples.

4. Activity Calculation

Unit definition: The amount of enzyme that produces 1 μmol NO_2^- per gram of soil sample per day is defined as one S-NR activity unit.

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

$$\text{S-NR (μmol/d/g soil sample)} = 0.5 \times (A_{assay\ tube} - A_{control\ tube}) \div (A_{standard\ tube} - A_{blank\ tube})$$

$C_{standard}$	Standard tube concentration, 0.1 μmol/mL
$V_{total\ reaction}$	Total reaction system volume, 0.3 mL
T	Reaction time, 1 d
W	Sample mass, 0.06 g

Precautions

1. Before formal measurement, select 2-3 samples with large expected differences for a preliminary test.

2. Instruments and supplies required but not provided: microplate reader, water bath, benchtop centrifuge, adjustable pipettes, 96-well plate, and distilled water.
3. A 100T kit can test 48 samples.

Visual Reference