Soil Nitrite Reductase(S-NiR) Activity Assay Kit

Product Code: 67054

Soil Nitrite Reductase Activity Assay Kit - Microplate Method

Product Code

67054

Product Introduction

Soil nitrite reductase is one of the key enzymes in denitrification. It participates in the reduction of nitrite to NO, and its activity reflects nitrogen conversion efficiency during biodegradation. This assay provides a basis for studying nitrogen transformation patterns in soil.

Nitrite reductase reduces NO_2^- to NO, decreasing the amount of NO_2^- available for the diazotization reaction that forms a purplish-red compound. The change in absorbance at 540 nm reflects soil nitrite reductase activity.

Example Measurement Data

Sample: forest soil.

Measurement	OD540 nm
No soil	0.915
Blank 1	0.051
Control	0.082
Assay	0.774 / 0.779

Actual readings may vary depending on the detection instrument and conditions. The data above are for reference only.

Package Contents and Storage

Kit size: 100T.

Code	Component	Quantity	Storage
67054.1	Reagent I	2.5 mL	2-8°C, protected from light
67054.2	Reagent II	0.05 g	2-8°C
67054.3	Reagent III	5 mL	2-8°C
67054.4	Reagent IV	8 mL	2-8°C, protected from light
67054.5	Reagent V	8 mL	2-8°C, protected from light
67054.6	Standard	1 mL	2-8°C, protected from light
67054.m	Manual	1 copy	-

Quality and Safety Information

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

Transportation and Storage

Transportation: this product is transported with ice packs.

Storage: store each component according to the instructions above. Shelf life: 180 days.

Instructions for Use

1. Sample Processing

Naturally air-dry fresh soil samples or dry them in a 37°C oven, then pass them through a 30-50 mesh sieve.

2. Reagent Preparation

- Reagent II: before use, add 5 mL distilled water to dissolve. Store at 4°C for up to 2 weeks or at -20°C for up to one year. After use, promptly return to 4°C or -20°C storage.
- Reagent III: if precipitation occurs, heat at 60-80°C to dissolve.
- Reagent IV: if precipitation occurs, heat at 60-80°C to dissolve.
- Standard: 10 μmol/mL sodium nitrite.
- Working solution: before use, mix Reagent IV and Reagent V at a 1:1 ratio according to the required amount. Prepare fresh before use. After preparation, it is valid for 48 h at 4°C.

3. Operating Procedure

1. Preheat the microplate reader for at least 30 min. Set the wavelength to 540 nm and zero with distilled water.
2. Dilute the 10 μmol/mL standard solution with distilled water to prepare 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0078125, and 0.001953125 μmol/mL standard solutions.

Standard Solution Dilution

No.	Concentration Before Dilution (μmol/mL)	Standard Solution Volume (μL)	Distilled Water Volume (μL)	Concentration After Dilution (μmol/mL)
1	10	40	360	1
2	1	200	200	0.5
3	0.5	200	200	0.25
4	0.25	200	200	0.125
5	0.125	200	200	0.0625
6	0.0625	200	200	0.03125
7	0.03125	100	300	0.0078125
8	0.0078125	100	300	0.001953125

In the following experiment, each standard tube requires 70 μL standard solution. Do not measure absorbance directly at this dilution step.

4. Measurement Procedure

Component	Soil-Free Tube	Blank Tube 1	Control Tube	Measurement Tube	Standard Tube	Blank Tube 2
Air-dried soil sample (g)	-	-	0.02	0.02	-	-
Distilled water (μL)	-	40	40	-	-	-
Reagent I (μL)	40	-	-	40	-	-
Reagent II (μL)	40	40	40	40	-	-
Mix thoroughly, then react at 25°C for 1 h.						
Reagent III (μL)	40	40	40	40	-	-
Shake thoroughly for 30 s, then centrifuge at 10,000 rpm and 4°C for 10 min.						
Supernatant (μL)	70	70	70	70	-	-

Standard (μL)	-	-	-	-	70	-
Distilled water (μL)	-	-	-	-	-	70
Working solution (μL)	140	140	140	140	140	140

Mix thoroughly, then transfer 200 μL reaction solution to a 96-well plate. Measure absorbance at 540 nm and record the values as $A_{soil\text{-}free}$, $A_{blank\ 1}$, $A_{control}$, $A_{measurement}$, $A_{standard}$, and $A_{blank\ 2}$.

Calculate: $\Delta A_{measurement} = (A_{soil\text{-}free} - A_{blank\ 1}) - (A_{measurement} - A_{control})$.

Calculate: $\Delta A_{standard} = A_{standard} - A_{blank\ 2}$.

> The soil-free tube, blank tube 1, and standard curve only need to be run 1-2 times.

Calculation

1. Standard Curve Preparation

Use the standard tube concentration (X, μmol/mL) and absorbance (Y, $\Delta A_{standard}$) to establish the standard curve. Substitute $\Delta A_{measurement}$as Y into the standard curve equation to calculate the sample concentration X (μmol/mL).

2. S-NiR Activity Calculation

Definition of enzyme activity unit: the amount of enzyme required to reduce 1 μmol NO_2^-per day per gram of soil sample is one enzyme activity unit.

S-NiR (U/g) = $X \times V_{reaction} \div W \div T = 2.88 \times X \div W$

- X: sample concentration calculated from the standard curve, μmol/mL
- $V_{reaction}$: total enzymatic reaction volume, 0.12 mL
- T: reaction time, 1 h = 1/24 d
- W: sample mass, g

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for a preliminary test. This 100T kit can test 48 samples.
2. Required instruments and supplies: balance, microplate reader, 96-well plate, adjustable pipette, desktop centrifuge, oven, 30-50 mesh sieve, constant-temperature water bath or incubator, and distilled water.
3. If $\Delta A_{measurement} > 2.5$, dilute the sample before measurement and modify the calculation formula accordingly.
4. The linear detection range of this kit is 0.001953125-1 μmol/mL.

Appendix

The standard curve is more accurate when prepared by the customer. Follow the measurement procedure above. Use either the standard curve formula or the absorbance values from each standard well to prepare a standard curve with $R^2 \geq 0.99$, then use the resulting equation for sample calculation.