Soil Nitrite Reductase(S-NiR) Activity Assay Kit

Product Code: 67052

Soil Nitrite Reductase Activity Assay Kit - Micro Method

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 transformation efficiency during biodegradation.

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

Reference Measurement Data

Sample: forest soil. $OD_{540\ nm}$: 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 detection conditions. The data above are for reference only.

Product Information

Product Code	67052
Size	100T
Shelf Life	180 days
Transportation	Transport with ice packs.

Package Contents and Storage

Code	Component	Amount	Storage Conditions
67052.1	Reagent I	2.5 mL	2-8°C, protected from light
67052.2	Reagent II	0.05 g	2-8°C
67052.3	Reagent III	5 mL	2-8°C
67052.4	Reagent IV	8 mL	2-8°C, protected from light
67052.5	Reagent V	8 mL	2-8°C, protected from light
67052.6	Standard	1 mL	2-8°C, protected from light
67052.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	--	--
Standard	--	--

Instructions for Use

1. Sample Processing

Air-dry fresh soil samples naturally or in a 37°C oven, then pass the dried samples 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 1 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, the working solution is valid for 48 h at 4°C.

3. Instrument Preparation

1. Preheat the visible spectrophotometer or microplate reader for at least 30 min.
2. Set the wavelength to 540 nm.
3. Zero the instrument with distilled water.

4. Standard Dilution

Dilute the 10 μmol/mL standard solution to prepare 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0078125, and 0.001953125 μmol/mL standard solutions for testing.

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 during this dilution step.

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 and react at 25°C for 1 h.						
Reagent III (μL)	40	40	40	40	-	-
Shake thoroughly for 30 s, then centrifuge at 10000 rpm, 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. Transfer 200 μL reaction solution into a micro glass cuvette or 96-well plate. Measure absorbance at 540 nm and record

the values as $A_{soil\text{-}free}$, $A_{blank\ 1}$, $A_{control}$, A_{assay}, $A_{standard}$, and $A_{blank\ 2}$.

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

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

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

Calculation

1. Standard Curve Preparation

Prepare the standard curve using the standard tube concentration as X, in μmol/mL, and absorbance as Y, where $Y = \Delta A_{standard}$. Substitute ΔA, where $Y = \Delta A_{measured}$, into the standard curve formula to calculate the sample concentration X, in μmol/mL.

2. S-NiR Activity Calculation

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

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

- X: sample concentration, 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 formal measurement, select 2-3 samples with large expected differences for a preliminary test. This 100T kit can test 48 samples.
2. Required instruments and supplies are not provided: balance, visible spectrophotometer or microplate reader, micro glass cuvette or 96-well plate, adjustable pipette, benchtop 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

For greater accuracy, prepare a standard curve using the provided operating procedure. The standard curve formula may be used, or a curve may be prepared from the absorbance values of each standard well obtained according to the operating table. Use a standard curve with $R^2 \geq 0.99$ to calculate sample results.