Soil Nitrite Reductase(S-NiR) Activity Assay Kit

Product Code: 67051

Soil Nitrite Reductase Activity Assay Kit - Spectrophotometric Method

Product Information

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

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

Reference example: Sample: forest soil. $OD_{540\ nm}$: soil-free tube 1.508; blank tube 1: 0.005; control tube: 0.043; measurement tube: 1.143/1.159.

Actual readings may vary depending on the testing instrument and test conditions. These data are for reference only.

Package Contents and Storage

Code	Component	Quantity	Storage Conditions
67051.1	Reagent 1	6 mL	2-8°C, protect from light
67051.2	Reagent 2	0.12 g	2-8°C
67051.3	Reagent 3	12 mL	2-8°C
67051.4	Reagent 4	25 mL	2-8°C, protect from light
67051.5	Reagent 5	25 mL	2-8°C, protect from light
67051.6	Standard	1 mL	2-8°C, protect from light
67051.m	Instructions	1 copy	-

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent 1	--	--
Reagent 2	--	--
Reagent 3	--	--
Reagent 4	--	--
Reagent 5	--	--
Standard	--	--

Transportation and Storage

Transportation: This product is transported with ice packs.

Storage: Store according to the instructions in this manual. Shelf life: 180 days.

Instructions for Use

1. Sample Processing

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

2. Reagent Preparation

- **Reagent 2:** Before use, add 12 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 store at 4°C or -20°C.
- **Reagent 3:** If precipitation occurs, heat at 60-80°C to dissolve.
- **Reagent 4:** If precipitation occurs, heat at 60-80°C to dissolve.
- **Standard:** 10 μmol/mL sodium nitrite.
- **Working solution:** Before use, mix Reagent 4 and Reagent 5 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. Operating Procedure

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

Standard Solution Dilution

Serial No.	Concentration Before Dilution (μmol/mL)	Standard Solution Volume (μL)	Distilled Water Volume (μL)	Concentration After Dilution (μmol/mL)
1	10	50	950	0.5
2	0.5	500	500	0.25
3	0.25	500	500	0.125
4	0.125	500	500	0.0625
5	0.0625	500	500	0.03125
6	0.03125	250	750	0.0078125
7	0.0078125	250	750	0.001953125

In the following experiment, each standard tube requires 350 μL standard solution. Do not directly measure absorbance 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)	-	-	0.1	0.1	-	-
Distilled water (μL)	-	200	200	-	-	-
Reagent I (μL)	200	-	-	200	-	-
Reagent II (μL)	200	200	200	200	-	-
Mix thoroughly and react at 25°C for 1 h.						
Reagent III (μL)	200	200	200	200	-	-
Shake thoroughly for 30 s. Centrifuge at 10000 rpm and 4°C for 10 min.						
Supernatant (μL)	350	350	350	350	-	-
Standard (μL)	-	-	-	-	350	-
Distilled water (μL)	-	-	-	-	-	350
Working solution (μL)	700	700	700	700	700	700

Mix thoroughly. Pipette 1 mL reaction solution into a 1 mL glass cuvette and measure absorbance at 540 nm. Record the readings as $A_{no\ soil}$, $A_{blank\ 1}$, $A_{control}$, $A_{measurement}$, $A_{standard}$, 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 performed 1-2 times.

5. Calculation

5.1 Standard Curve

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

5.2 S-NiR Activity

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

S-NiR (U/g) = $X \times V_{reaction} \div W \div T = 14.4 \times X \div W$

- **$V_{reaction}$:** total volume of enzymatic reaction, 0.6 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 pre-experiment. This 50T kit can test 24 samples.
2. Required instruments and supplies to be prepared by the user: balance, visible spectrophotometer, 1 mL glass cuvette, 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 0.001953125-0.5 μmol/mL.

Appendix

The standard curve should be prepared by the customer for greater accuracy. Refer to the operating table above for the steps. The customer may use the standard curve formula or use the absorbance values of each standard well obtained according to the operating table to prepare a standard curve with $R^2 \geq 0.99$, then obtain the calculation formula for sample calculation.