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# Soil nitrite reductase (S-NiR) Assay Kit

Note: Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

Catalog Number: AK0163

**Size**:50T/24S

# **Components:**

Reagent 1: Powder×1, storage at 4°C. Dissolve with 1mL of distilled water before use. The reagent can be saved for 2 weeks at 4°C. Dilute 400 times with distilled water before use.

Reagent 2: Powder $\times$ 1, storage at 4°C . Dissolve with 15mL of distilled water before use. The reagent can be saved for 2 weeks at 4°C .

Reagent 3:15 mL×1, storage at 4°C. This solution is a saturated solution, just use the supernatant

Reagent 4: 25 mL×1, storage at RT and protected from light.

Reagent 5: 25 mL×1, storage at RT and protected from light.

Standard: 1 mL×1, storage at 4°C . 10 μmol/mL ofNaNO<sub>2</sub> standard solution.

## **Product Description:**

Soil nitrite reductase (S-NiR) is one of the key enzymes in denitrification. It is a reductase produced by soil denitrifying bacteria. It can reduce  $NO_2$  to NO. The activity reflects the conversion efficiency of nitrogen in the process of biodegradation, and provides a certain basis for the study of nitrogen conversion.

Nitrite reductase can reduce  $NO_2$ - to NO, and reduce the  $NO_2$ - in the sample to participate in the diazotization reaction to produce a purple-red compound, that is, the change in absorbance at 540nm can reflect the activity of nitrite reductase in soil.

### **Reagents and Equipment Required but Not Provided:**

Spectrophotometer, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, 1mL glass cuvette, sieve (30-50 mesh, or smaller), ice and distilled water.

## **Procedure:**

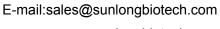
## I. Sample preparation

Fresh soil samples are naturally air-dried or oven dried at 37°C and sieved through 30-50 mesh.

#### **I**. Determination

- 1. Preheat spectrophotometer for 30min, adjust the wavelength to 540 nm and set the counter to zero with distilled water.
- 2. Dilute the standard solution with distilled water to prepare  $0.8 \times 0.6 \times 0.4 \times 0.2 \times 0.1 \times 0.05 \,\mu\text{mol/mL}$  standard solution.
- 3. Add reagent to a 1.5mL EP tube:

	Non-matrix	Blank tube1	Control tube	Test tube	Standard	Blank tube
	tube (An)	(Ab1)	(Ac)	(At)	tube (As)	(Ab)
sample (g)	-	-	0.1	0.1	-	-
Distilled water	_	200	200	_	_	_
(µL)						





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	T					
Reagent 1 (µL)	200		-	200	_	_
Reagent 2 (µL)	200	200	200	200	-	_
Reagent 3 (µL)	200	200	200	200	_	_
Fully shak	-	-				
Supernatant	400	400	400	400	_	_
( µ L )						
Standard (µL)	_	-	-	-	400	_
Reagent 4 (µL)	400	400	400	400	400	400
Reagent 5 (µL)	400	400	400	400	400	400
Distilled water	300	300	300	300	300	700
( µ L )						

Mix well and react at room temperature for 15min. The absorbance at the wavelength of 540nm, and record them as An, Ab1, Ac, At, As and Ab, and calculate  $\Delta A = (An-Ab1)-(At-Ac)$ ,  $\Delta As = As-Ab$ . Nonmatrix tube (An), Blank tube1 (Ab1), Blank tube (Ab) only need to be done 1-2 times.

### III. Calculation

1. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis,  $\Delta As$  as Y-axis. Take  $\Delta A$  into the equation to obtain x ( $\mu mol/mL$ )

#### 2. Fermentation broth:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the reduction of  $1 \mu mol NO_2$ - per day every gram soil in the reaction system.

S-NiR (U/g) =
$$x \times Vr \div T \div W = 4.8 \times x \div W$$
.

T: reaction time, 3h=1/8 d;

V1: Enzymatic reaction volume, 0.6mL;

W: soil weight, g;

## **Related Products:**

AK0161/AK0160 Soil Hydroxylamine Reductase Activity Assay Kit

AK0329/AK0328 Soil Lignin peroxidase(S-Lip) Activity Assay Kit

AK0118/AK0117 Soil β- 1,4-Glucanase Activity Assay Kit

AK0120/AK0119 Soil Leucine Arylamidase (S-LAP) Activity Assay Kit

AK0574/AK0573 Soil Saccharase(S-SC) Activity Assay Kit

AK0370/AK0369 Soil Nitrate Reductase (NR) Activity Assay Kit