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# Soil Nitrate Reductase (NR) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Cat No:** AK0370 **Size:** 50T/24S

# **Components:**

Reagent I:  $30ml \times 1$ , storage at -20°C. Reagent II:  $5ml \times 1$ . Storage at -20°C.

Reagent  ${\rm I\hspace{-.1em}I\hspace{-.1em}I}$ : 5ml×1. Storage at 4°C .

Reagent IV:10ml×1. Storage at -20°C.

Reagent V:25ml×1. Storage at 4°C. Dissolves at 60°C if crystallization appeared.

Reagent VI: 25ml×1. Storage at 4°C.

Standard: 1ml×1, 10µmol/mL sodium nitrite. Storage at -20°C.

Preparation of standard solution: Dilute standard to  $0.8 \times 0.6 \times 0.4 \times 0.2 \times 0.1 \mu mol/mL$  with deionized water

#### **Product Description:**

S-NR catalyzes the reduction of nitrate to nitrite in soil, which is the key enzyme of nitrate reduction in soil. Study on the activity of S-NR is of great significance for rational fertilization and reduction of nitrogen loss.

S-NR catalyzes the reduction of nitrate to nitrite,  $NO_3^-+NADH^+H^+\rightarrow NO_2^-+NAD^++H_2O$ ; the generated nitrite can quantitatively generate red azo compounds with p-aminobenzenesulfonic acid and  $\alpha$ -naphthylamine under acidic conditions; The unreacted NADH will inhibit the subsequent color reaction, and then carry out the subsequent reaction with PMS; the generated red azo compounds are 520 nm has a maximum absorption peak, which can be determined by spectrophotometry.

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

Spectrophotometer, water bath, low temperature centrifuge, 1ml glass cuvette, distilled water, 30 mesh sieve (or smaller), ice and distilled water.

#### **Procedure:**

## I. Sample handling:

The fresh soil sample shall be dried by natural air or dried in 37°C oven, and it shall be passed through 30-50 meshes.

- 1. Preheat the spectrophotometer 30min, adjust wavelength to 520 nm, set zero with distilled water.
- 2. Add reagents with the following list:

1.5mL tube
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	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)	
Air-dried soil (g)	0.1	0.1			
NaNO <sub>2</sub> Standard (μL)			100		
distilled water (µL)	100	100		100	
Reagent I (μL)	365	365	365	365	
Reagent II (µL)	35		35	35	
Mix thoroughly, incubate at 37°C for 24 h					
Reagent <b>II</b> (μL)	50	50	50	50	
Reagent II (µL)		35			
Mix immediately, and centrifuge at 8000rpm for 5min at RT					
Supernatant (μL)	400	400	400	400	
Reagent <b>IV</b> (μL)	100	100	100	100	
Mix thoroughly, incubate at 37°C for 20min					
Reagent V (μL)	250	250	250	250	
Reagent VI (µL)	250	250	250	250	

Mix thoroughly and then measure the absorption of 520nm after 20min. Calculate  $\triangle$   $A_T$ =  $A_T$ - $A_C$ ,  $\triangle$   $A_S$ = $A_S$ - $A_B$ . Standard tube and Blank tube just need test once or twice and each test tube should set a control.

# **Ⅲ.** S-NR Activity Calculation

- 1. Make standard curve: Get the standard curve according to standard concentration (x) and  $\triangle A_S(y)$ . y=kx+b. Take  $\Delta A_T$  into the formula to get the concentration ( $\mu$ mol/mL) of sample(x)
- 2. Unit definition: one unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of  $1\mu$ mol of  $NO_2^-$  every 1g of soil in one day.

NR 
$$(U/g) = x \times V_S \div W \div T = 0.1x \div W$$

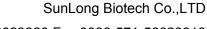
V<sub>S</sub>: standard volume, 0. 1mL;

W: the weight of air-dried soil;

T: time, 1d.

#### Note:

- 1 、 Reagent II, Reagent II , Reagent IV put on ice before use and put into -20°C as soon as use d up.
- 2 . Each test tube is provided with a control tube.
- 3 \ If  $\Delta A$  is less than 0.01 \, please prolong the reaction time(37°C water bath time).
- 4  $\searrow$  When  $\Delta A$  is greater than 1, the supernatant can be diluted with distilled water, and th en measured, multiplying the dilution times in the calculation formula.



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## **Related Products:**

Glutaminase (GLS) Activity Assay Kit AK0436/AK0435

Glutamic Acid Dehydrogenase (GDH) Activity Assay Kit AK0434/AK0433

Nitrate Reductase (NR) Activity Assay Kit AK0301/AK0300