SUNLONG
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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×1, storage at -20℃ .

Reagent Ⅱ: 5ml×1. Storage at -20℃ .

Reagent Ⅲ: 5ml×1. Storage at 4℃ .

Reagent Ⅳ:10ml×1. Storage at -20℃ .

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

Reagent Ⅵ: 25ml×1. Storage at 4℃ .

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

Preparation of standard solution: Dilute standard to 0.8 、 0.6 、 0.4 、 0.2 、 0. 1μmol/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 α-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℃ 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

	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Air-dried soil （g）	0.1	0.1		
$NaNO_2$ 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℃ for 24 h				
Reagent Ⅲ （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 Ⅳ （μL）	100	100	100	100
Mix thoroughly, incubate at 37℃ 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 △ A_T= A_T-A_C， △ 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 △A_S (y). y=kx+b. Take ΔA_T into the formula to get the concentration (μmol/mL) of sample(x)

2. Unit definition: one unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1μmol ofNO_2^- every 1g of soil in one day.

NR （U/g） =x×V_S÷W÷T=0. 1x÷W

V_S： standard volume, 0. 1mL;
W： the weight of air-dried soil;
T： time, 1d.

Note:

1、 Reagent I, Reagent Ⅱ, Reagent Ⅳ put on ice before use and put into -20℃ as soon as used up.

2、 Each test tube is provided with a control tube.

3、 If ΔA is less than 0.01， please prolong the reaction time(37℃ water bath time).

4、When ΔA is greater than 1, the supernatant can be diluted with distilled water, and then measured, multiplying the dilution times in the calculation formula.

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Related Products:

AK0436/AK0435 Glutaminase (GLS) Activity Assay Kit
AK0434/AK0433 Glutamic Acid Dehydrogenase (GDH) Activity Assay Kit
AK0301/AK0300 Nitrate Reductase (NR) Activity Assay Kit