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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/ Microplate reader

Cat No: AK0369

Size： 100T/48S

Components:

Reagent I: 25 ml×1, storage at -20℃ .

Reagent Ⅱ: 5 ml×1. Storage at -20℃ .

Reagent Ⅲ: 5 ml×1. Storage at 4℃ .

Reagent Ⅳ: 5 ml×1. Storage at -20℃ .

Reagent Ⅴ:10 ml×1. Storage at 4℃ . Dissolves at 60℃ if crystallization appeared.

Reagent Ⅵ: 10 ml×1. Storage at 4℃ .

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

Preparation of standard solution: dilute standard to 1, 0.8, 0.6, 0.4, 0.2μmol/mL with distilled 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/microplate reader, water bath, centrifuge, adjustable pipette, 30 mesh sieve (or smaller), micro glass cuvette/96 well flat-bottom plate, ice and distilled water.

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.

Procedure:

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

	1.5mL EP tube			
	Test tube(T)	Control tube (C)	Standard Tube (S)	Blank tube (B)
Air-dried soil （g）	0.05	0.05		
$NaNO_2$ Standard （μL）			50	
distilled water （μL）	50	50		50
Reagent I （μL）	180	180	180	180
Reagent Ⅱ （μL）	18		18	18
Mix thoroughly, incubate at 37℃ for 24h				
Reagent Ⅲ （μL）	25	25	25	25
Reagent Ⅱ （μL）		18		
Mix immediately， and centrifuge at 8000rpm for 5min at RT				
Supernatant （μL）	80	80	80	80
Reagent Ⅳ （μL）	20	20	20	20
Mix thoroughly, incubate at 37℃ for 20min				
Reagent Ⅴ （μL）	50	50	50	50
Reagent Ⅵ （μL）	50	50	50	50

Mix thoroughly and then measure the absorbance of 520nm after 20min. Calculate △ A_T= A_T-A_C，△

A_S=A_S-A_B.

S-NR activity Calculation

1. Make standard curve: Taking 1, 0.8, 0.6, 0.4, 0.2μmol/mL standard solution as the X-axis, the A_T as the Y-axis, draw the standard curve. Gain a linear regression equation, y=kx+b. Take △A_S 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=0.05x÷W

V_S： standard volume, 0.05 mL
W： Air-dried soil, g
T： time, 1d

Note:

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