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: BC3105

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:

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	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 $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$.

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 ΔA_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 of NO_2^- every 1g of soil in one day.

   NR（U/g）$= x \times V_S \div W \div T = 0.05x \div W$

   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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