Soil Nitrate Reductase (NR) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC3100

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