Soil nitrite reductase (S-NiR) Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC2990

Size:50T/24S

Components:

Reagent 1: Powder×1, storage at 4℃. Dissolve with 1mL of distilled water before use. The reagent can be saved for 2 weeks at 4℃. Dilute 400 times with distilled water before use.

Reagent 2: Powder×1, storage at 4℃. Dissolve with 15mL of distilled water before use. The reagent can be saved for 2 weeks at 4℃.

Reagent 3:15 mL×1, storage at 4℃. This solution is a saturated solution, just use the supernatant

Reagent 4: 25 mL×1, storage at RT and protected from light.

Reagent 5: 25 mL×1, storage at RT and protected from light.

Standard: 1 mL×1, storage at 4℃. 10 μmol/mL of $NaNO_2$ standard solution.

Product Description:

Soil nitrite reductase (S-NiR) is one of the key enzymes in denitrification. It is a reductase produced by soil denitrifying bacteria. It can reduce NO_2^- to NO. The activity reflects the conversion efficiency of nitrogen in the process of biodegradation, and provides a certain basis for the study of nitrogen conversion.

Nitrite reductase can reduce NO_2^- to NO, and reduce the NO_2^- in the sample to participate in the diazotization reaction to produce a purple-red compound, that is, the change in absorbance at 540nm can reflect the activity of nitrite reductase in soil.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, 1mL glass cuvette, sieve (30-50 mesh, or smaller), ice and distilled water.

Procedure:

I. Sample preparation

Fresh soil samples are naturally air-dried or oven dried at 37℃ and sieved through 30-50 mesh.

II. Determination

1. Preheat spectrophotometer for 30min, adjust the wavelength to 540 nm and set the counter to zero with distilled water.
2. Dilute the standard solution with distilled water to prepare 0.8、0.6、0.4、0.2、0.1、0.05 μmol/mL standard solution.
3. Add reagent to a 1.5mL EP tube:

	Non-matrix tube (An)	Blank tube1 (Ab1)	Control tube (Ac)	Test tube (At)	Standard tube (As)	Blank tube (Ab)
sample（g）	-	-	0.1	0.1	-	-
Distilled water（μL）	-	200	200	-	-	-

Reagent 1（μL）	200	-	-	200	-	-
Reagent 2（μL）	200	200	200	200	-	-
After mixing, react at 25℃ for 3 h						
Reagent 3（μL）	200	200	200	200	-	-
Fully shake for 30s, 10000rpm centrifuge for 10min at 4℃					-	-
Supernatant（μL）	400	400	400	400	-	-
Standard（μL）	-	-	-	-	400	-
Reagent 4（μL）	400	400	400	400	400	400
Reagent 5（μL）	400	400	400	400	400	400
Distilled water（μL）	300	300	300	300	300	700
Mix well and react at room temperature for 15min. The absorbance at the wavelength of 540nm, and record them as An, Ab1, Ac, At, As and Ab, and calculate ΔA = (An-Ab1)-(At-Ac), ΔAs = As-Ab. Non-matrix tube (An), Blank tube1 (Ab1), Blank tube (Ab) only need to be done 1-2 times.						

III. Calculation

1. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis, ΔAs as Y-axis. Take ΔA into the equation to obtain x (μmol/mL)
2. **Fermentation broth:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the reduction of 1μmol NO_2^- per day every gram soil in the reaction system.

S-NiR (U/g) =x×Vr ÷ T÷W=4.8×x÷ W.

T：reaction time, 3h=1/8 d;

V1: Enzymatic reaction volume, 0.6mL;

W: soil weight, g;

Related Products：

BC3010/BC3015 Soil Hydroxylamine Reductase Activity Assay Kit

BC1970/BC1975 Soil Lignin peroxidase(S-Lip) Activity Assay Kit

BC4030/BC4035 Soil β-1,4-Glucanase Activity Assay Kit

BC4020/BC4025 Soil Leucine Arylamidase (S-LAP) Activity Assay Kit

BC0240/BC0245 Soil Saccharase(S-SC) Activity Assay Kit

BC3100/BC3105 Soil Nitrate Reductase (NR) Activity Assay Kit