Soil nitrite reductase (S-NiR) Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC2995

Size:100T/48S

Components:

Reagent I: Powder×1, storage at 4℃. Dissolve with 1mL of distilled water before use. The reagent can be saved for 2 weeks at 4℃. Dilute 100 times with distilled water before use, prepared when the solution will be used.

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

Reagent III: Powder×1, storage at 4℃. Dissolve with 15mL of distilled water before use. This solution is a saturated solution, just use the supernatant. The reagent can be saved for 2weeks at 4℃.

Reagent IV: 15 mL×1, storage at RT and protected from light.

Reagent V: 15 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/ Microplate reader, low temperature desk centrifuge, water-bath, adjustable transferpettor, mortar, micro glass cuvette/ 96-well flat-bottom plate, 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/microplate reader for 30 min, adjust the wavelength to 540 nm and set spectrophotometer counter to zero with distilled water.

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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 1.5 mL EP tube:

Reagent	Non-matrix tube (An)	Blank tube1 (Ab1)	Control tube (Ac)	Test tube (At)	Standard tube (As)	Blank tube (Ab)
sample（g）	-	-	0.05	0.05	-	-
Distilled water（μL）	-	100	100	-	-	-
Reagent I（μL）	100	-	-	100	-	-
Reagent II（μL）	100	100	100	100	-	-
After mixing, react at 25℃ for 3 h.						
Reagent III（μL）	100	100	100	100	-	-
Fully shake for 30s, centrifuge at 10000 rpm for 10 min at 4℃.					-	-
Superatant（μL）	100	100	100	100	-	-
Standard（μL）	-	-	-	-	100	-
Reagent IV（μL）	100	100	100	100	100	100
Reagent V（μL）	100	100	100	100	100	100
Distilled water（μL）						100
Mix well and react at room temperature for 15min. Take 200 μL into a micro glass cuvette/96 well plate and measure the absorbance value 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=2.4×x÷ W.

T：reaction time, 3h=1/8 d;

V1: Enzymatic reaction volume, 0.3 mL;

W: soil weight, g;

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