Soil Hydroxylamine Reductase (S-HR) Assay Kit

Operation Equipment: Spectrophotometer

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

Catalog Number: NA0651

Size: 50T/24S

Components:

Reagent I: 15 mL×1. Storage at 4℃.

Reagent Ⅱ: Powder×1. Storage at 4℃. Add 15mL of distilled water to fully dissolve before use.

Reagent Ⅲ: 50 mL×1. Storage at 4℃.

Reagent Ⅳ: 30 mL×1. Storage at 4℃.

Reagent Ⅴ: 15 mL×1. Storage at 4℃.

Reagent Ⅵ: 10 mL×1. Storage at 4℃ and protected from light.

Reagent Ⅶ: 10 mL×1. Storage at 4℃ and protected from light.

Standard solution: powder×1, Storage at 4℃; Add 1.028 mL of distilled water with filly dissolve before use to prepare 140 μmol/mL Hydroxylamine hydrochloride standard for standby.

Product Description

Soil hydroxylamine reductase can reduce the intermediate product hydroxylamine formed in the process of soil nitrogen metabolism to ammonia, and the reduced compounds in the soil can be used as hydrogen donors. Its strength affects the ammonia volatilization loss of nitrogen in the process of soil nitrogen metabolism, and indirectly affects the utilization efficiency of nitrogen.

Fe^{3+} in ammonium ferric sulfate can oxidize hydroxylamine to nitrogen and reduce itself to Fe^{2+}, Fe^{2+} forms orange red complex with o-phenanthroline under weak acid condition, orange red complex has absorption peak at 510nm. hydroxylamine reductase acts on hydroxylamine that could reduce the amount of formation of complex, and the decrease of absorption value at 510nm can reflect the activity of hydroxylamine reductase.

Reagents and Equipment Required but Not Provided

Spectrophotometer, scales, centrifuge, transferpettor, 1 mL glass cuvette, vortex shaker, nitrogen blower, EP tube**,** sieve (30-50 mesh) and distilled water.

Procedure

1. **Sample preparation:**
   Fresh soil samples are naturally air-dried or oven dried at 37℃ and passed through a 30-50 mesh sieve.
2. **Determination steps and sample adding table:**

a. Preheat spectrophotometer more than 30 min, adjust wavelength to 510 nm and set zero with distilled

water.

b. Dilute the 140 μmol/mL standard solution to 4.375、2.1875 、1.094 、0.547 、0.2735 、0.13675μmol/mL of standard solution.

c. Operate according to the following table:

	Control tube	Test tube	Matrix free tube	Standard tube	Blank tube
Drying soil（g）	0.1	0.1	-	-	-
Reagent I（μL）	-	200	200	-	-
standard solution（μL）	-	-	-	200	-
Distilled water（μL）	200	-	-	-	200
Reagent II（μL）	200	200	200	200	200
Reagent III（μL）	600	600	600	600	600
After mixing, use N_2 air flow to remove the air in the tube, seal immediately, and react at 30 ℃ for 1h.					
Reagent IV（μL）	400	400	400	400	400
Full shaking for 10min, centrifugation at 8000rpm and 25℃ for 10 min.					
supernatant（μL）	100	100	100	100	100
Reagent V（μL）	200	200	200	200	200
Reagent VI（μL）	100	100	100	100	100
Reagent VII（μL）	100	100	100	100	100
Distilled water（μL）	500	500	500	500	500
Mix well, let it stand at 25℃ for 10 minutes, measure the absorbance value at 510 nm in the 1 mL glass cuvette, and record it as A_C, A_T, A_M, A_S and A_B. Calculate $\triangle A = (A_M - A_B) - (A_T - A_C)$, $\triangle A_S = A_S - A_B$. Each test tube needs to be provided with a control tube, Matrix free tube and blank tube needs to be done 1-2 times.					

Calculation of S-HR activity:

1. The regression equation determined under standard conditions is y=kx+b; x is the concentration of standard substance (mg/mL), y is the absorption value. Take ΔA into the equation to get x (mg/mL).

2. Calculation of HR activity:

Unit definition: one unit is defined as an enzyme activity that enzyme catalyzes the production of 1 μmol of hydroxylamine per day every gram soil.

The activity of S-HR（U/g soil）$=x \times V_{RI} \div W \div T = 4.8x \div W$

V_{RI}: the volume of add Reagent I, 0.2 mL;

W: sample weight, g;

T: reaction time: 1/24h.

Note

1. The dissolved oxygen concentration in the surface layer of the soil is large, and the soil below 5cm in the surface layer should be taken for sampling, otherwise the enzyme activity is low or cannot be measured.

2. Reagent Ⅲ should not be left open as much as possible. Cover and tighten immediately after taking it out. If it is left open for a long time, it can be cooled to normal temperature by using a boiling water bath for 10 minutes (cover).
3. When △A is greater than 0.8, it is recommended to dilute the sample supernatant before measuring.
4. It is best to use a nitrogen blower to remove the dissolved oxygen in the reaction system. If there is no such device, seal it immediately after adding reagent Ⅲ and react at 30 ° C for 1 hour.

Experimental example:

1. Take 2 tubes of 0.02 g clover soil, operate according to the determination steps, use 96 well plate to measure and calculate $\Delta A = (A_M - A_B) - (A_T - A_C)$ =0.603-（ 0.499-0.139） =0.243, standard curve: y=0.1693x+0.0083, x = 1.3863, S-HR activity calculated according to soil weight:
S-HR activity (U/g soil sample) =4.8×x÷W=4.8×1.3863÷0.1=66.542 U/g soil sample.
2. Take 2 tubes of 0.02 g forest soil, operate according to the determination steps, use 96 well plate to measure and calculate $\Delta A = (A_M - A_B) - (A_T - A_C)$ =0.603-（ 0.543-0.199） =0.259, standard curve: y=0.1693x+0.0083, x = 1.4808
S-HR activity (U/g soil sample) =4.8×x÷W=4.8×1.4808÷0.1=71.078 U/g soil sample.

Related Products：

NA0653/NA0412 Soil Nitrite Reductase Activity Assay Kit
NA0728/NA0486 Soil Lignin peroxidase(S-Lip) Activity Assay Kit
NA0361/NA0360 Soil β-1,4-Glucanase Activity Assay Kit
NA0371/NA0362 Soil Leucine Arylamidase(S-LAP) Activity Assay Kit