# Soil Hydroxylamine Reductase (S-HR) Assay Kit

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

**Operation Equipment:** Spectrophotometer/ Microplate reader

**Catalog Number:** NA0410

**Size:** 100T/48S

**Components:**

Reagent I: 5 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃. Add 5 mL of distilled water to fully dissolve before use.

Reagent III: 15 mL×1. Storage at 4℃.

Reagent IV: 10 mL×1. Storage at 4℃.

Reagent V: 5 mL×1. Storage at 4℃.

Reagent VI: 2.5 mL×1. Storage at 4℃.

Reagent VII: 2.5 mL×1. Storage at 4℃.

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/ Microplate reader, scales, centrifuge, transferpettor, micro glass cuvette/ 96-well flat-bottom plate, 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/ microplate reader 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） | 0.02 | 0.02 | - | - | - |
| Reagent I（μL） | - | 40 | 40 | - | - |
| standard solution（μL） | - | - | - | 40 | - |
| Distilled water（μL） | 40 | - | - | - | 40 |
| Reagent II（μL） | 40 | 40 | 40 | 40 | 40 |
| Reagent III（μL） | 120 | 120 | 120 | 120 | 120 |
| 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） | 80 | 80 | 80 | 80 | 80 |
| Full shaking for 10min, centrifugation at 8000rpm and 25℃ for 10min. | | | | | |
| supernatant（μL） | 20 | 20 | 20 | 20 | 20 |
| Reagent V（μL） | 40 | 40 | 40 | 40 | 40 |
| Reagent VI（μL） | 20 | 20 | 20 | 20 | 20 |
| Reagent VII（μL） | 20 | 20 | 20 | 20 | 20 |
| Distilled water（μL） | 100 | 100 | 100 | 100 | 100 |
| Mix well, let it stand at 25℃ for 10 minutes, measure the absorbance value at 510 nm in the micro glass cuvette/96 well plate, 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. | | | | | |

**3.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）$=x \times V_{RI} \div W \div T$

$V_{RI}$: the volume of add Reagent I, 0.04 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 5 cm 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 ΔA is greater than 0.5, 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℃ 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 $\Delta A = (A_M - A_B) - (A_T - A_C) = 0.38 - (0.326-0.116) = 0.170$, standard curve: $y = 0.0923x + 0.0138$, $x = 1.6923$, S-HR activity calculated according to soil weight:
S-HR activity (U/g soil sample) = $x \times 0.04 \div W \div T = 1.6923 \times 0.04 \div 0.02 \times 24 = 81.23$ 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 ΔA = (AM-AB) - (AT-AC) = 0.38 - (0.316-0.086) = 0.150, standard curve: $y = 0.0923x + 0.0138$, $x = 1.4756$
S-HR activity (U/g soil sample) = $x \times 0.04 \div W \div T = 1.4756 \times 0.04 \div 0.02 \times 24 = 70.829$ U/g soil sample.

**Related Products：**

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|---|---|
| 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 |