

Soil Neutral Phosphatase (S-NP) Assay Kit

Note: Take two or three different samples for prediction before test. Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: AK0545 Size: 100T/96S

Components:

Reagent I: Liquid 42 mL×1. Storage at 4°C . Protect from light.

Reagent II : Powder×1. Storage at 4°C . Dissolve with 100 mL of distilled water before use.

Reagent Ⅲ: Liquid 5 mL×1. Storage at 4°C.

Reagent IV: Powder×1 bottle. Storage at 4°C and protect from light. Dissolve with 576 μ L of absolute ethyl alcohol (requird but not provided) and 24 μ L of distilled water before use. Do not use any more if it turns brown,

Standard: Liquid 1 mL×1. Storage at 4°C . 0.5 µmol/mL Phenol standard solution.

Product Description:

Soil phosphatase is an enzyme which catalyzes soil organic phosphate mineralization, the activity influences the decomposition and transformation of organic phosphate and its bio-availability directly, which is the indicator of evaluating the direction and intensity of soil phosphorus bio-transformation. Soil phosphatase is influenced by the content of carbon, nitrogen, available phosphorus in the soil and pH. Soil phosphatase is divided into three types: acidic, neutral and alkaline phosphatase according to the optimum

pH.

In neutral condition, soil neutral phosphatase (S-NP) can catalyzes the hydrolysis of disodium phenyl phosphate to produce phenol and disodium hydrogen phosphate, the activity of S-NP can be calculate by detecting the content of phenol.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, micro cuvette/96 well flat-bottom plate, 37°C constant temperature incubator, desk centrifuge, adjustable pipette, analytical balance, toluene, alcohol, ice and distilled water.

Procedure:

I. Crude enzyme preparation:

Add 0.05 mL of toluene into 0.1 g of dry soil sample, shake slightly for 15 min, add 0.4 mL of Reagent I, mix thoroughly and keep in 37° C constant temperature incubator for 24 h, then add 1 mL of Reagent



I quickly to stop the catalysis, mix thoroughly, Centrifuge at 10000 rpm for 10 minutes at 25°C to remove insoluble materials, and take the supernatant on ice before test.

II. Determination procedure:

1. Preheat spectrophotometer or microplate reader for 30 minutes, adjust the wavelength to 660 nm, set zero with distilled water.

2. Blank tube: Take a micro glass cuvette or 96 well flat-bottom plate, add to 10 μ L of Reagent I, 40 μ L of Reagent III, 4 μ L of Reagent IV, mix thoroughly. After coloring, add 146 μ L of distilled water, mix thoroughly. Place it at room temperature for 30 min. Detect the absorbance at 660 nm, record as A_B.

3. Standard tube: Take a micro glass cuvette or 96 well flat-bottom plate, add to 10 μ L of standard, 40 μ L of Reagent III, 4 μ L of Reagent IV, mix thoroughly. After coloring, add 146 μ L of distilled water, mix thoroughly. Place it at room temperature for 30 min. Detect the absorbance at 660 nm, record as A_S.

4. Test tube: Take a micro glass cuvette or 96 well flat-bottom plate, add to 10 μ L of supernatant, 40 μ L of Reagent III, 4 μ L of Reagent IV, mix thoroughly. After coloring, add to 146 μ L of distilled water, mix thoroughly. Place it at room temperature for 30 min. Detect the absorbance at 660 nm, record as A_T. **Note:** Blank tube and standard tube only need to test once or twice.

III. S-NP activity calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol of phenol per day at 37°C every gram of soil sample.

S-ACP (nmol/d/g)=[C×(A_T-A_B) \div (A_S-A_B)]×Vrv \div W \div T

 $=725\times(A_T-A_B)\div(A_S-A_B)\div W$

C: Standard concentration, 0.5 µmol/mL;

Vrv: Total volume in catalyze system, 1.45 mL;

W: Soil sample weight, g;

T: Reaction time, 24 hours=one day;

Related products:

AK0592/AK0591	Soil Urease(UE) Activity Assay Kit
AK0594/AK0593	Soil Polyphenoloxidase Activity Assay Kit
AK0586/AK0585	Soil β -glucosidase (β - GC) Activity Assay Kit
AK0508/AK0507	Soil Peroxidase Activity Assay Kit