

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: BC0465

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 µL of absolute ethyl alcohol (required but not provided) and 24 µL 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 catalyze the hydrolysis of disodium phenyl phosphate to produce phenol and disodium hydrogen phosphate, the activity of S-NP can be calculated 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 II 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 μL of Reagent I, 40 μL of Reagent III, 4 μL of Reagent IV, mix thoroughly. After coloring, add 146 μL 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 μL of standard, 40 μL of Reagent III, 4 μL of Reagent IV, mix thoroughly. After coloring, add 146 μL 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 μL of supernatant, 40 μL of Reagent III, 4 μL of Reagent IV, mix thoroughly. After coloring, add to 146 μL 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.

$$\begin{aligned} \text{S-ACP (nmol/d/g)} &= [C \times (A_T - A_B) \div (A_S - A_B)] \times V_{rv} \div W \div T \\ &= 725 \times (A_T - A_B) \div (A_S - A_B) \div W \end{aligned}$$

C: Standard concentration, 0.5 $\mu\text{mol/mL}$;

V_{rv} : Total volume in catalyze system, 1.45 mL;

W: Soil sample weight, g;

T: Reaction time, 24 hours=one day;

Related products:

- BC0120/BC0125 Soil Urease(UE) Activity Assay Kit
- BC0110/BC0115 Soil Polyphenoloxidase Activity Assay Kit
- BC0160/BC0165 Soil β -glucosidase (β - GC) Activity Assay Kit
- BC0890/BC0895 Soil Peroxidase Activity Assay Kit