Soil Neutral Phosphatase (S-NP) Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC0460

Components:

Reagent I: Liquid 21 mL×1. Storage at 4℃. Protect from light.

Reagent II: powder×1. Storage at 4℃. Dissolved with 50 mL of distilled water before use.

Reagent III: Liquid 11 mL×1. Storage at 4℃.

Reagent IV: Powder×1 bottle. Storage at 4℃ and protect from light. Dissolved with 1152 μL of absolute ethyl alcohol (provide for oneself) and 48 μL of distilled water before use. Do not use any more if it turns brown,

Standard: Liquid 1 mL×1. Storage at 4℃. 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, 37℃ constant temperature incubator, desk centrifuge, adjustable pipette, 1mL glass cuvette, analytical balance, toluene, alcohol, ice and distilled water.

Procedure:

I. Crude enzyme preparation:

Add 0.05 mL of toluene to 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℃ constant temperature incubator for 24 h, then add 1 mL of Reagent II quickly to stop the catalysis, mix thoroughly. Centrifuge at 8000 rpm for 10 minutes at 25℃ to remove insoluble materials, and take the supernatant on ice before test.

II. **Determination procedure:**

1. Preheat Spectrophotometer for 30 minutes, adjust the wavelength to 660 nm, set zero with distilled water.

2. Blank tube: Take a 1 mL glass cuvette, add to 50 μL of Reagent I, 200 μL of Reagent III, 20 μL of Reagent IV, mix thoroughly. After coloring, add to 730 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 1 mL glass cuvette, add to 50 μL of standard, 200 μL of Reagent III, 20 μL of Reagent IV, mix thoroughly. After coloring, add to 730 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 1 mL glass cuvette, add to 50 μL of supernatant, 200 μL of Reagent III, 20 μL of Reagent IV, mix thoroughly. After coloring, add to 730 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-ACP 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℃ every gram of soil sample.

$S\text{-}ACP(nmol/d/g)=[C\times(A_T-A_B)\div(A_S-A_B)]\times Vrv\times 1000\div W\div T$

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

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;

1000: Unit conversion factor, 1 μmol=1000 nmol.

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