

## Soil Neutral Phosphatase (S-NP) Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** AK0546

### Components:

**Reagent I:** Liquid 21 mL×1. Storage at 4°C . Protect from light.

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

**Reagent III:** Liquid 11 mL×1. Storage at 4°C .

**Reagent IV:** Powder×1 bottle. Storage at 4°C 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°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, 37°C 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°C 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°C 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  $\mu$ L of Reagent I, 200  $\mu$ L of Reagent III, 20  $\mu$ L of Reagent IV, mix thoroughly. After coloring, add to 730  $\mu$ 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 1 mL glass cuvette, add to 50  $\mu$ L of standard, 200  $\mu$ L of Reagent III, 20  $\mu$ L of Reagent IV, mix thoroughly. After coloring, add to 730  $\mu$ 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 1 mL glass cuvette, add to 50  $\mu$ L of supernatant, 200  $\mu$ L of Reagent III, 20  $\mu$ L of Reagent IV, mix thoroughly. After coloring, add to 730  $\mu$ 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-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°C every gram of soil sample.

$$\text{S-ACP}(\text{nmol/d/g}) = [C \times (A_T - A_B) \div (A_S - A_B)] \times V_{rv} \times 1000 \div W \div T$$
$$= 725 \times (A_T - A_B) \div (A_S - A_B) \div W$$

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

$V_{rv}$ : 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  $\mu$ mol=1000 nmol.

### Related products:

AK0592/AK0591 Soil Urease(UE) Activity Assay Kit

AK0594/AK0593 Soil Polyphenoloxidase Activity Assay Kit

AK0586/AK0585 Soil  $\beta$ -glucosidase ( $\beta$ -GC) Activity Assay Kit

AK0508/AK0507 Soil Peroxidase Activity Assay Kit