

## Soil Alkaline Phosphatase (S-AKP/ALP) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer/ Microplate Reader

**Catalog Number:** BC0285

**Size:** 100T/96S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 42 mL ×1	2-8°C
Reagent II	Powder × 1	2-8°C
Reagent III	Liquid 2.5 mL ×1	2-8°C
Reagent IV	Powder × 2	2-8°C
Standard	Liquid 1 mL ×1	2-8°C

### Solution Preparation:

1. Reagent II: Before use, add 100 mL distilled water to dissolve fully and store at 2-8°C for 8 weeks.
2. Reagent IV: Before of use, take 1 branch and add 576  $\mu$ L anhydrous ethanol (Required but not provided), and dissolve 24  $\mu$ L distilled water fully. Store unused reagents at 2-8°C for 1 week (can not be used after Browning).
3. Standard: 0.5 $\mu$ mol/mL phenol solution.

### Product Description:

Soil phosphatase is an enzyme which catalyzes soil organic phosphate mineralization, the activity level influence the decomposition and transformation of organic phosphate and its bio-availability directly, which is the index 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, its divided into three types: acidic, neutral and alkaline according to the optimum pH.

In alkaline condition, soil alkaline phosphatase (S-AKP/ALP) can catalyzes disodium phenyl phosphate to form phenol and disodium hydrogen phosphate, the activity of S-AKP/ALP can be calculated by detecting the content of phenol.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, desk centrifuge, transferpettor, Water bath/constant temperature incubator, analytical balance, mortar, (express delivery not allowed), alcohol, ice and distilled water.

### Determination Procedure:

#### Sample processing

1. The fresh soil sample is air-dried naturally or in an oven at 37°C, and passed through a 30-50 mesh

sieve.

2. Weigh about 0.1 g of air-dried mixed soil, add 0.05 mL of toluene (provided by yourself), shake gently for 15 minutes; add 0.4 mL of reagent once and shake well, place it in a 37°C water bath/constant temperature incubator, start timing, catalyze Reaction for 24h; when the time is up, quickly add 1mL reagent II and mix well to stop the reaction catalyzed by the enzyme. Centrifuge at 10000 rpm and 25°C for 10 min, and place the supernatant on ice for testing.

### Measurement procedure

1. Preheat Spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 660 nm, Spectrophotometer set zero with distilled water.
2. Blank tube: Take micro glass cuvette/96 well plate, add 10 µL of Reagent I, 20 µL of Reagent III, 4 µL of Reagent IV, mix thoroughly, add 166 µL of distilled water after color development. Mix thoroughly. Allow to stand for 30 minutes at room temperature. Determine the absorbance at 660 nm and record as  $A_B$ .
3. Standard tube: Take micro glass cuvette/96 well plate, add 10 µL of standard solution, 20 µL of Reagent III, 4 µL of Reagent IV, mix thoroughly, add 166 µL of distilled water after color development. Mix thoroughly. Allow to stand for 30 minutes at room temperature. Determine the absorbance at 660 nm and record as  $A_S$ .
4. Test tube: Take micro glass cuvette/96 well plate, add 10 µL of supernatant, 20 µL of Reagent III, 4 µL of Reagent IV, mix thoroughly, add 166 µL of distilled water after color development. Mix thoroughly. Allow to stand for 30 minutes at room temperature. Determine the absorbance at 660 nm and record as  $A_T$ .

**Note:** blank tube and standard tube just need to test once or twice.

### S-AKP/ALP activity calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of phenol in the reaction system per day(24 hours) at 37°C every gram soil sample.

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

C: Concentration of standard solution, 0.5 µmol/mL;

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

W: Sample weight, g;

T: Reaction time, 24 h=1 day;

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

### Note:

The linear range is 0.03125 µmol/mL-2.5 µmol/mL.

### Recent Product Citations:

[1] Liu B, Wang S, Wang J, et al. The great potential for phytoremediation of abandoned tailings pond using ectomycorrhizal *Pinus sylvestris*[J]. *Science of The Total Environment*, 2020, 719: 137475.

[2] Shao T, Zhao J J, Liu A, et al. Effects of soil physicochemical properties on microbial communities in different ecological niches in coastal area[J]. *Applied Soil Ecology*, 2020: 103486.

**Related Products:**

- BC0120/BC0125 Soil Urease(UE) Activity Assay Kit
- BC0110/BC0115 Soil Polyphenoloxidase Activity Assay Kit
- BC0160/BC0165 Soil  $\beta$ -glucosidase( $\beta$ - GC) Activity Assay Kit