

Soil Acid Phosphatase (S-ACP) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC0145

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 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: Dissolve with 100 mL of distilled water before use. The reagent can be stored for 8 weeks at 2-8°C.
2. Reagent IV: Before use, add 576 μL of ethanol (required but not provided) and 24 μL of distilled water to one bottle and fully dissolve it. The reagents can be stored at 2-8°C for 2 weeks. (One bottle of reagent is enough for 100T, another is to prolong the stable storage time of the reagent. The reagent can't be used after turning brown).
3. Standard: 0.5 μmol/mL phenol standard solution.

Product Description:

Soil phosphatase is an enzyme which catalyze soil organic phosphate mineralization, the activity influence directly the decomposition and transformation of organic phosphate and its bioavailability. The activity is the indicator of evaluating the direction and intensity of soil phosphorus biotransformation. Soil phosphatase is influenced by the content of carbon, nitrogen, available phosphorus in the soil and pH. Soil phosphatase is divided into 3 types: acidic, neutral and alkaline phosphatase according to the optimum pH.

In acidic condition, soil acid phosphatase (S-ACP) can hydrolyze disodium phenyl phosphate to phenol and disodium hydrogen phosphate. The activity of S-ACP can be calculated by measuring the amount of phenol produced.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath/constant temperature incubator, analytical balance, centrifuge, transferpettor, micro glass cuvette/96 well plate, 30-50 mesh sieve, toluene (>98%, AR), ethanol (>98%, AR), ice and distilled water.

Procedure:

I. 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 (required but not provided), shake gently for 15 minutes; add 0.4 mL of reagent I 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 for 10 min at 25°C and place the supernatant on ice for testing.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 660 nm, set spectrophotometer zero with distilled water.
2. Blank tube: Take a micro glass cuvette/96 well flat-bottom plate, add 10 µL of **Reagent I**, 40 µL of Reagent III, 4 µL of Reagent IV, mix thoroughly. Then add 146 µL of distilled water after color development. Mix thoroughly and place for 30 minutes at room temperature. Determine the absorbance at 660 nm and record as A_B. The blank tube only needs to be measured 1-2 times.
3. Standard tube: Take a micro glass cuvette/96 well flat-bottom plate, add 10 µL of **standard solution**, 40 µL of Reagent III, 4 µL of Reagent IV, mix thoroughly. Then add 146 µL of distilled water after color development. Mix thoroughly and place for 30 minutes at room temperature. Determine the absorbance at 660 nm and record as A_S. The standard tube only needs to be measured 1-2 times.
4. Test tube: Take a micro glass cuvette/96 well flat-bottom plate, add 10 µL of **supernatant**, 40 µL of Reagent III, 4 µL of Reagent IV, mix thoroughly. Then add 146 µL of distilled water after color development. Mix thoroughly and place for 30 minutes at room temperature. Determine the absorbance at 660 nm and record as A_T.

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 in the reaction system per day every gram soil sample.

$$\begin{aligned} \text{S-ACP activity (U/g weight)} &= [C \times (A_T - A_B) \div (A_S - A_B)] \times V_{TV} \times 1000 \div W \div T \\ &= 725 \times (A_T - A_B) \div (A_S - A_B) \div W \end{aligned}$$

C: Standard concentration, 0.5 µmol/mL;

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

W: Soil sample weight, g;

T: Reaction time, 24 hours=1 day;

1000: 1 µmol=1000 nmol.

Recent Protect Citations:

- [1] Muhammad I, Yang L, Ahmad S, Zeeshan M, Farooq S, Khan A, Zhou XB. Irrigation and Nitrogen Fertilization Alter Soil Bacterial Communities, Soil Enzyme Activities, and Nutrient Availability in Maize Crop. *Front Microbiol.* 2022 Feb 3; 13:833758. doi: 10.3389/fmicb.2022.833758.

- [2] Pu Q, Zhang K, Poulain AJ, Liu J, Zhang R, Abdelhafiz MA, Meng B, Feng X. Mercury drives microbial community assembly and ecosystem multifunctionality across a Hg contamination gradient in rice paddies. *J Hazard Mater.* 2022 Aug 5; 435:129055. doi: 10.1016/j.jhazmat.2022.129055. Epub 2022 May 4. PMID: 35650726.
- [3] Feng L, Gao Z, Ma H, He S, Liu Y, Jiang J, Zhao Q, Wei L. Carbonate-bound Pb percentage distribution in agricultural soil and its toxicity: Impact on plant growth, nutrient cycling, soil enzymes, and functional genes. *J Hazard Mater.* 2023 Jun 5; 451:131205. doi: 10.1016/j.jhazmat.2023.131205. Epub 2023 Mar 13. PMID: 36934701.
- [4] Rao G, Yan SZ, Song WL, Lin D, Chen YJ, Chen SL. Distribution, assembly, and interactions of soil microorganisms in the bright coniferous forest area of China's cold temperate zone. *Sci Total Environ.* 2023 Nov 1;897:165429. doi: 10.1016/j.scitotenv.2023.165429. Epub 2023 Jul 10. PMID: 37437627.

References:

- [1] Guan S. *Soil Enzyme and Its Research Method* [M]. Beijing: Science Press, 1982.
- [2] Powell MEA, Smith MJH. The Determination of Serum Acid and Alkaline Phosphatase Activity with 4-Aminoantipyrine (A.A.P.) [J]. *Journal of Clinical Pathology*, 1954, 7: 245-248.
- [3] Belfield A, Goldberg DM. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine[J]. *Enzyme*, 1971, 12(5): 561-573.

Related Products:

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| BC0280/BC0285 | Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit |
| BC0110/BC0115 | Soil Polyphenoxidase Activity Assay Kit |
| BC0120/BC0125 | Soil Urease (S-UE) Activity Assay Kit |