

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

Catalog Number: BC0140

Size: 50T/48S

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 21 mL×1	2-8°C
Reagent II	Powder ×1	2-8°C
Reagent III	Liquid 11 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 50 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. (The reagent can't be used it 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 contents 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 Equipments Required but Not Provided:

Spectrophotometer, water bath/constant temperature incubator, centrifuge, transferpettor, 1 mL glass cuvette, analytical balance, mortar, 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 8000 rpm for 10 min at 25°C and place the supernatant on ice for testing.

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 50 μ L of **Reagent I**, 200 μ L of Reagent III, 20 μ L of Reagent IV, mix thoroughly. Then add 730 μ L 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 1 mL glass cuvette, add 50 μ L of **standard solution**, 200 μ L of Reagent III, 20 μ L of Reagent IV, mix thoroughly. Then add 730 μ L 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 1 mL glass cuvette, add 50 μ L of **supernatant**, 200 μ L of Reagent III, 20 μ L of Reagent IV, mix thoroughly. Then add 730 μ L 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.

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

C: Standard concentration, 0.5 μ mol/mL;

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

W: Soil sample weight, g;

T: Reaction time, 24 hours=1 day;

1000: 1 μ mol=1000 nmol.

Recent Product Citations:

- [1] Ahsan T, Tian PC, Gao J, Wang C, Liu C, Huang YQ. Effects of microbial agent and microbial fertilizer input on soil microbial community structure and diversity in a peanut continuous cropping

system. J Adv Res. 2023 Nov 28: S2090-1232(23)00367-3. doi: 10.1016/j.jare.2023.11.028. Epub ahead of print. PMID: 38030126.

- [2] Wang S, Song M, Wang C, Dou X, Wang X, Li X. Mechanisms underlying soil microbial regulation of available phosphorus in a temperate forest exposed to long-term nitrogen addition. Sci Total Environ. 2023 Dec 15;904:166403. doi: 10.1016/j.scitotenv.2023.166403. Epub 2023 Aug 18. PMID: 37597553.
- [3] Yang C, Xia L, Zeng Y, Chen Y, Zhang S. Hexaploid *Salix rehderiana* is more suitable for remediating lead contamination than diploids, especially male plants. Chemosphere. 2023 Aug; 333:138902. doi: 10.1016/j.chemosphere.2023.138902. Epub 2023 May 12. PMID: 37182717.
- [4] Bian X, Yang X, Zhang K, Zhai Y, Li Q, Zhang L, Sun X. Potential of *Medicago sativa* and *Perilla frutescens* for overcoming the soil sickness caused by ginseng cultivation. Front Microbiol. 2023 Apr 5; 14:1134331. doi: 10.3389/fmicb.2023.1134331. PMID: 37089541; PMCID: PMC10113677.

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 Polyphenoloxidase (S-PPO) Activity Assay Kit |
| BC0120/BC0125 | Soil Urease (S-UE) Activity Assay Kit |