Soil Acid Phosphatase (S-ACP) Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer/ Microplate Reader
Catalog Number: BC0145
Size:100T/96S

Components:
Reagent I: Liquid 42 mL×1, storage at 4℃ and protect from light.
Reagent II: Powder×1, storage at 4℃. Dissolve with 100 mL of distilled water before use.
Reagent III: Liquid 5 mL×1 bottle, storage at 4℃.
Reagent IV: Powder×1, storage at 4℃. Dissolve with 576 μL of absolute ethyl alcohol (required but not provided) and 24 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, storage at 4℃.

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 bio-availability. The activity 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 divideded into three 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, micro glass cuvette/96 well flat-bottom plate, 37℃ incubator, centrifuge, table centrifuge, transferpettor, analytical balance, toluene, alcohol, ice and distilled water.

Procedure:

I. Crude enzyme extraction:

Add 0.05 mL of methylbenzene to 0.1 g of dry soil sample, shake slightly for 15 minutes. Add 0.4 mL of Reagent I, mix thoroughly and keep in 37℃ incubator for 24 hours. Add 1 mL of Reagent II immediately and mix thoroughly to stop the catalysis. Centrifuge at 10000 rpm for 10 minutes at room temperature, take the supernatant on ice for testing.

II. Determination procedure:

1. Preheat Spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 660 nm, set 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.

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.

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.

Note: Blank tube only need to be tested 1-2 times.

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.

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

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=1 day;

1000: 1 μmol=1000 nmol.

Recent Protect 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] Hou Q, Wang W, Yang Y, et al. Rhizosphere microbial diversity and community dynamics during potato cultivation[J]. European Journal of Soil Biology, 2020, 98: 103176.

References:

[1] 关松荫.土壤酶及其研究法[M].北京：科学出版社，1982.

Related Products:

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