Soil Neutral Phosphatase (S-NP) Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0465

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℃
Reagent II	Powder×1	2-8℃
Reagent III	Liquid 5 mL×1	2-8℃
Reagent IV	Powder×2	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. **Reagent II:** Dissolved with 100 mL of distilled water before use, unused reagents can be stored at 2-8℃ for 8 weeks.
2. **Reagent IV:** Dissolved with 576 μL of absolute ethyl alcohol (self-supplied reagent) and 24 μL of distilled water before use. Do not use any more if it turns brown, unused reagents can be stored at 2-8℃ for 2 weeks.
3. **Standard:** 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.

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Visible spectrophotometer/microplate reader, micro glass cuvette/96 well plate, desk centrifuge,

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water bath/constant temperature incubator, balance, adjustable pipette, mortar, alcohol (>98%, AR), toluene (>98%, AR), 30-50 mesh sieve, ice and distilled water.

Procedure:

I. Sample preparation:

1. Fresh soil samples are naturally air-dried or oven to dry at 37℃, then sieved by 30 ~ 50 mesh sieve.
2. Weigh about 0.1g of dry soil sample, add 0.05mL of toluene (self-supplied reagent), and gently shake for 15 minutes; Then add 0.4mL of Reagent I and shake well, place it in a constant temperature incubator at 37℃, start timing, and catalyze the reaction for 24 hours; After that, take out and quickly add 1mL of reagent II, mix thoroughly to terminate the enzyme catalyzed reaction. Centrifuge at 10000rpm and 25 ℃ for 10 minutes, then take the supernatant and place it on ice for testing.

II. Determination procedure:

1. Preheat spectrophotometer or microplate reader for 30 min, adjust the wavelength to 660 nm, set zero with distilled water.
2. Blank tube: Take a micro glass cuvette or 96 well flat-bottom plate, add to 10 μL of Reagent I, 40 μL of Reagent III, 4 μL of Reagent IV, mix thoroughly. After coloring, add 146 μL 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 micro glass cuvette or 96 well flat-bottom plate, add to 10 μL of standard, 40 μL of Reagent III, 4 μL of Reagent IV, mix thoroughly. After coloring, add 146 μL 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 micro glass cuvette or 96 well flat-bottom plate, add to 10 μL of supernatant, 40 μL of Reagent III, 4 μL of Reagent IV, mix thoroughly. After coloring, add to 146 μL of distilled water, mix thoroughly. Place it at room temperature for 30 min. Detect the absorbance at 660 nm, record as A_T.

III. S-NP 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℃ every gram of soil sample.

S-NP activity (U/g Soil)=$[C\times(A_T-A_B)\div(A_S-A_B)]\times1000\times Vrv\div W\div T=725\times(A_T-A_B)\div(A_S-A_B)\div W$

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=one day;

Notes:

Please place the sample on ice during the testing period to avoid enzyme denaturation or inactivation.

Related publications:

[1] Li Z, Song Z, Qiu L, Cao Y, Gu H, Wang Z, Liu X, Qian X. Quantitative distribution and quantized ecological threat of microplastics in farmland: Shanghai as an example. J Hazard Mater. 2023

Nov 23; 465:133069. doi: 10.1016/j.jhazmat.2023.133069. Epub ahead of print. PMID: 38056264.

[2] Xu Y, Ding H, Zhang G, Li Z, Guo Q, Feng H, Qin F, Dai L, Zhang Z. Green manure increases
peanut production by shaping the rhizosphere bacterial community and regulating soil metabolites under continuous peanut production systems. BMC Plant Biol. 2023 Feb 1;23(1):69. doi: 10.1186/s12870-023-04079-0. PMID: 36726076.

[3] Zhang J, Cheng K, Liu X, Dai Z, Zheng L, Wang Y. Exogenous abscisic acid and sodium nitroprusside regulate flavonoid biosynthesis and photosynthesis of Nitraria tangutorum Bobr in alkali stress. Front Plant Sci. 2023 Mar 15; 14:1118984. doi: 10.3389/fpls.2023.1118984. PMID: 37008502; PMCID: PMC10057120.

[4] Xiao J, Lan S, Farías ME, Xia L, Song S. The living forms of Microcoleus vaginatus and their contributions to the aggregate structure of biocrusts. FEMS Microbiol Ecol. 2023 Apr 7;99(5): fiad040. doi: 10.1093/femsec/fiad040.

Related products:

[1] 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.

[2] Belfield A, Goldberg DM. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine[J]. Enzyme, 1971, 12(5): 561-573.

Related products:

BC0120/BC0125	Soil Urease (S-UE) Activity Assay Kit
BC0110/BC0115	Soil Polyphenoloxidase (S-PPO) Activity Assay Kit
BC0160/BC0165	Soil β-glucosidase (S-β- GC) Activity Assay Kit
BC0890/BC0895	Soil Peroxidase (S-POD) Activity Assay Kit

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