Soil Neutral Protease Activity Assay Kit

Note: The reagents of this product have changed, please pay attention to and strictly follow the instructions.

Operation Equipment: Spectrophotometer

Catalog Number: BC0270

Size: 50T/24S

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 20 mL×1	2-8℃
Reagent II	Powder×2	2-8℃
Reagent III	Liquid 12 mL×1	2-8℃
Reagent IV	Liquid 40 mL×1	2-8℃
Reagent V	Liquid 10 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. Reagent II: Dissolve with 6 mL of Reagent I one of the bottle before using, stir and dissolve in boiling water bath, and unused liquid can be stored at 2-8℃ for 4 weeks.

2.Standard: 20μmol/mL tyrosine solution.

Product Description:

Soil protease is involved in the transformation of amino acids, proteins and other organic compounds containing protein nitrogen in soil. Those hydrolytic product is one of the nitrogen sources of higher plants. Soil neutral protease catalyzes the hydrolysis of protein in neutral environment, which is related to soil organic matter content, nitrogen and other soil properties. Under neutral conditions, soil neutral protease could hydrolyze casein to produce tyrosine. In alkaline conditions, tyrosine reduced phosphomolybdic acid compound to form tungsten blue with absorbance peak in 680 nm.

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

Reagents and Equipment Required but Not Provided:

visible spectrophotometer, mortar/water bath/constant temperature incubator, adjustable pipette, desk centrifuge, mortar, 1 mL glass cuvette, distilled water, 30~50 mesh sieve.

Procedure

I Sample preparation: (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

The fresh soil is dried naturally or dried in the oven at 37℃, then sieved by 30 ~ 50 mesh sieve.

II Determination Procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 680 nm and set zero with distilled water.
2. Dilution of standard solution: dilute 20 μmol/mL tyrosine standard solution with distilled water 100 times to 0.2 μmol/mL for use
3. Sample determination

Reagent	Test tube (A_T)	Contrast tube (A_C)	Standard tube (A_S)	Blank tube (A_B)
Sample (g)	0.1	0.1	-	-
Reagent I (μL)	100	100	-	-
Reagent II (μL)	200	-	-	-
Mix thoroughly, react for 24 hours at 37℃. During the reaction, shake 5-6 times to mix the soil sample and the reaction solution thoroughly.			-	-
Reagent III (μL)	200	200	-	-
Reagent II (μL)	-	200	-	-
Mix thoroughly, centrifuge at 10000 rpm for 10 minutes at room temperature, take supernatant.			-	-
Supernatant (μL)	220	220	-	-
Standard (μL)	-	-	220	-
Distilled water (μL)	-	-	-	220
Reagent IV (μL)	650	650	650	650
Reagent V (μL)	130	130	130	130
Mix thoroughly, incubate at 40℃ for 10 minutes, centrifuge at 10000 rpm for 10 minutes at room temperature. Take the supernatant to detect the absorbance at 680 nm, record as A_T, A_C, A_S, A_B. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$. (The blank and standard tubes only need to be measured 1-2 times, a control tube is required for each assay tube).				

III Calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 μmol tyrosine in the reaction system per day (24 hours) every gram soil sample.

Soil neutral protease(U/g) = $C_S \times \Delta A_T \div \Delta A_S \times V_{RT} \div W \div T = 0.1 \times \Delta A_T \div \Delta A_S \div W$.

C_S: The concentration of standard tube, 0.2 μmol/mL;

V_{RT}: Total volume of reaction system, 0.5 mL;

T: Reaction time, 1 day=24 hours;

W: Sample weight, g.

Note:

If absorbance value>1, the sample can be determined after appropriately diluted., multiply dilution times when calculate.

Experimental example:

1. Two parts of 0.1 g of clover soil are put into 1.5 mL EP tube as contrast tube and test tube respectively. According to the determination procedure, the results showed that $\Delta A_T=A_T-A_C$ = 0.320-0.218 = 0.102, $\Delta A_S = A_S-A_B$= 0.540-0.028 = 0.512

Soil neutral protease (U/g soil sample) = $0.1\times\Delta A_T \div A_S \div W = 0.1 \times 0.102 \div 0.512 \div 0.1$ = 0.1992 U/g soil sample.

2. Two parts of 0.1g of forest soil are taken into 1.5 mL EP tube, which is control tube and test tube respectively. According to the determination steps, $\Delta A_T= A_T-A_C$= 0.195-0.135 = 0.06, $\Delta A_S = A_S-A_B$ = 0.540-0.028 = 0.512

Soil neutral protease (U/g soil sample) = $0.1 \times \Delta A_T \div A_S \div W = 0.1 \times 0.06 \div 0.512 \div 0.1$ = 0.1172 U/g soil sample.

Recent Product Citations:

[1] Wang X, Wang Q, Li W, Zhang D, Fang W, Li Y, Wang Q, Cao A, Yan D. Long-term effects of chloropicrin fumigation on soil microbe recovery and growth promotion of Panax notoginseng. Front Microbiol. 2023 Jul 14;14:1225944. doi: 10.3389/fmicb.2023.1225944. PMID: 37520348; PMCID: PMC10375714.

[2] Xiao J, Lan S, Farías ME, Qian L, Xia L, Song S, Wu L. 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. PMID: 37028939.

[3] Manyun Zhang, Jun Wang, Shahla Hosseini Bai, et al. Evaluating the effects of phytoremediation with biochar additions on soil nitrogen mineralization enzymes and fungi. Environmental Science and Pollution Research. May 2018;(IF2.914)

[4] Zhang M, Wang W, Wang J, et al. Dynamics of biochemical properties associated with soil nitrogen mineralization following nitrification inhibitor and fungicide applications[J]. Environmental Science and Pollution Research, 2017, 24(12): 11340-11348.

References:

[1] Huang X, Tian B, Niu Q. et al. An extracellular protease from Brevibacillus laterosporus G4 without parasporal crystals can serve as a pathogenic factor in infection of nematodes[J]. Research in Microbiology, 2005, 156(5-6): 719-727.

[2] Daniel Geisseler, William R Horwath. Regulation of extracellular protease activity in soil in response to different sources and concentrations of nitrogen and carbon [J]. Soil Biology and Biochemistry, 2008, 40(12): 3040-3048.

[3] Zhu W, Luan H, Bu Y. et al. Changes in taste substances during fermentation of fish sauce and the correlation with protease activity [J]. Food Research International, 2021, 144:110349.

Related Products:

BC0860/BC0865	Soil Acid Protease Activity Assay Kit
BC0880/BC0885	Soil Alkaline Protease Activity Assay Kit
BC0280/BC0285	Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
BC0110/BC0115	Soil Polyphenoloxidase Activity Assay Kit
BC4040/BC4045	Soil Neutral Invertase(S-NI) Activity Assay Kit
BC4030/BC4035	Soil β-1,4-Glucanase Activity Assay Kit
BC4010/BC4015	Soil β-Xylosidase(S-β-XYS) Activity Assay Kit

Flow chart:

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