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Soil Neutral Protease Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Microplate reader

Cat No: AK0567

Size: 100T/48S

Components:

Reagent I: Liquid 20 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃. Add 10 mL of Reagent I before use. Mix thoroughly in boiled water for reserve, set aside.

Reagent III: Powder×1.Storage at 4℃. Add 10 mL of distilled water before use and dissolve thoroughly, set aside.

Reagent IV: Liquid 20 mL×1.Storage at 4℃.

Reagent V: Liquid 5 mL×1.Storage at 4℃.

Standard: Liquid 1 mL×1, 20 μmol/mL Tyrosine solution. Storage at 4℃.

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.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, methylbenzene, distilled water, 50 mesh sieve(or smaller).

Procedure:

I Sample preparation:

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

II Determination

1. Preheat spectrophotometer or microplate reader for 30 minutes, adjust the wavelength to 680 nm, 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

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Reagent	Test tube (A_T)	Contrast tube (A_C)	Standard tube (A_S)	Blank tube (A_B)
Sample (g)	0.05	0.05	-	-
Reagent I (μL)	50	50	-	-
Reagent II (μL)	100	-	-	-
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)	100	100	-	-
Reagent II (μL)	-	100	-	-
Mix thoroughly, centrifuge at 10000 rpm for 10 minutes at room temperature, take supernatant.				
Supernatant (μL)	44	44	-	-
Standard (μL)	-	-	44	-
Distilled water (μL)	-	-	-	44
Reagent IV (μL)	130	130	130	130
Reagent V (μL)	26	26	26	26
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. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$.				

Note: Standard tube and blank tube only need to be measured once or twice. A control tube is provided for each test tube.

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)= $C_S \times \Delta A_T \div \Delta A_S \times V_{RT} \div W \div T = 0.05 \times \Delta A_T \div \Delta A_S \div W$.

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

V_{RT} : Total volume of reaction system, 0.25 mL;

T: Reaction time, 1 day=24 hours;

W: Sample weight, g.

Note:

If absorbance value>1.5, the sample can be determined after being 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 $\Delta A_T = A_T - A_C = 0.582 - 0.355 = 0.227$, $\Delta A_S = A_S - A_B = 0.374 - 0.045 = 0.329$

Soil neutral protease (U/g soil sample) = $0.05 \times \Delta A_T \div A_S \div W = 0.05 \times 0.227 \div 0.329 \div 0.05 = 0.69$ 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, $\Delta A_T = A_T - A_C = 0.23 - 0.117 = 0.053$, $\Delta A_S = A_S - A_B = 0.374 - 0.045 = 0.329$

Soil neutral protease (U/g soil sample) = $0.1 \times \Delta A_T \div A_S \div W = 0.05 \times 0.053 \div 0.329 \div 0.05 = 0.1611$ U/g soil sample.

Recent Product Citations：

[1] 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)

[2] 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.

Related Products：

AK0512/AK0511	Soil Acid Protease Activity Assay Kit
AK0510/AK0509	Soil Alkaline Protease Activity Assay Kit
AK0566/AK0565	Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
AK0594/AK0593	Soil Polyphenoloxidase Activity Assay Kit
AK0116/AK0115	Soil Neutral Invertase(S-NI) Activity Assay Kit
AK0118/AK0117	Soil β- 1,4-Glucanase Activity Assay Kit
AK0122/AK0121	Soil β-Xylosidase(S-β-XYS) Activity Assay Kit