

Soil Neutral Protease Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: NA0847

Size: 50T/24S

Components:

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

Reagent II: Powder×1. Storage at 4°C. Add 10 mL of Reagent I before use. Stir and dissolve in boiling water bath, set aside.

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

Reagent IV: Liquid 40 mL×1. Storage at 4°C.

Reagent V: Liquid 10 mL×1. Storage at 4°C.

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

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, water bath, transferpeltor, 1 mL glass cuvette, methylbenzene, distilled water, 50 mesh sieve(or smaller).

Procedure

I Sample preparation:

The fresh soil is dried naturally or dried in the oven at 37°C, 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°C. 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°C 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 contrast 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.

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

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] 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 :

NA0805/NA0563	Soil Acid Protease Activity Assay Kit
NA0804/NA0374	Soil Alkaline Protease Activity Assay Kit
NA0846/NA0604	Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
NA0862/NA0619	Soil Polyphenoloxidase Activity Assay Kit
NA0373/NA0372	Soil Neutral Invertase(S-NI) Activity Assay Kit
NA0361/NA0360	Soil β -1,4-Glucanase Activity Assay Kit
NA0364/NA0363	Soil β -Xylosidase(S- β -XYS) Activity Assay Kit