

Soil Acid Protease Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Cat No: NA0805

Size: 50T/24S

Components:

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

Reagent II: Power×1, store at 4°C. Add 10 mL of Reagent VI before use. Mix thoroughly in boiled water for reserve.

Reagent III: Liquid 12 mL×1, store at 4°C.

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

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

Reagent VI: Liquid 15 mL×1, store at 4°C.

Standard: Liquid 1 mL×1, 20 µmol/mL tyrosine solution, store 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. Its hydrolysate is one of the nitrogen sources of higher plants. Soil acid protease catalyzes the hydrolysis of protein in acid environment, which is related to soil organic matter content, nitrogen and other soil properties. Under acid conditions, soil acid protease could hydrolyze casein to produce tyrosine. In alkaline condition, tyrosine reduced phosphomolybdic acid compound to form tungsten blue, which has an absorbance peak at 680 nm.

Required reagents and equipment:

Spectrophotometer, water-bath, adjustable pipette, 1 mL glass cuvette, methylbenzene, distilled water, 50 meshes sieve.

Protocol:

I. Sample preparation

The fresh soil is dried naturally or air dried at 37°C, then sieved by 30 ~ 50 meshes sieve.

II. Procedure:

1. Preheat spectrophotometer 30 minutes, adjust 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

Reagent name	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Sample (g)	0.1	0.1	-	-
Reagent I (μL)	200	200	-	-
Reagent II (μL)	100	-	-	-
Mix thoroughly and then react 24 hours at 37°C. During the reaction process, shake 5-6 times to help the soil sample contact with reagent thoroughly.				
Reagent III (μL)	200	200	-	-
Reagent II (μL)	-	100	-	-
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 supernatant and detect the absorbance at 680 nm, properly record 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. One control tube is provided for each test tube.

III. Calculation:

Definition: One unit of soil acid protease activity is defined as the amount of enzyme catalyzes the production of 1 μmol of tyrosine in every gram soil sample per day (24 hours)

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

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:

When the absorbance value is more than 1, it is suggested that the supernatant should be diluted and then determined. Attention should be paid to multiply the dilution multiple when calculating.

Experimental example:

1. Two parts of 0.1 g of soil samples are taken and put into 1.5 mL EP tube respectively as control tube and test tube. According to the determination procedure, the enzyme activity is calculated as follows: $\Delta A_T = A_T - A_C = 0.206 - 0.178 = 0.028$, $\Delta A_S = A_S - A_B = 0.540 - 0.028 = 0.512$

Soil acid protease (U/g soil sample) = $0.05 \times \Delta A_T \div A_S \div W = 0.1 \times 0.028 \div 0.512 \div 0.1 = 0.0547$ U/g soil sample.

2. Two parts of 0.1 g of forest soil are put into 1.5 mL EP tube as control tube and measuring tube respectively. According to the determination procedure, the enzyme activity was calculated as follows:

$$\Delta A_T = A_T - A_C = 0.831 - 0.612 = 0.219, \Delta A_S = A_S - A_B = 0.540 - 0.028 = 0.512$$

$$\text{Soil acid protease (U/g soil sample)} = 0.05 \times \Delta A_T \div A_S \div W = 0.1 \times 0.219 \div 0.512 \div 0.1 = 0.0313 \text{ U/g soil sample.}$$

Related products:

NA0847/NA0605 Soil Neutral Protease Activity Assay Kit

NA0804/NA0374 Soil Alkaline Protease Activity Assay Kit