

## Soil Alkaline Protease Activity Assay Kit

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

**Detection equipment:** Spectrophotometer

**Cat No:** BC0880

**Size:** 50T/24S

**Components:**

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

**Reagent II:** Powder×2, store at 4°C. Add 6 mL of Reagent I before use, mix thoroughly, and stir in boiling water and dissolve, unused reagents can be stored at 4°C for 4 weeks.

**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.

**Standard:** Liquid 1 mL×1, 20 μmol/mL tyrosine solution, store at 4°C.

**Description:**

Soil protease take part in the transform of amino acid, protein and other organic compounds contain protein nitrogen in soil. The products are one of the nitrogen sources in higher plants. Soil alkaline protease catalyzes hydrolysis of protein in alkaline condition which is related to soil organic content, nitrogen and other soil properties. In alkaline condition, soil alkaline protease can hydrolyze tyrosine to produce tyrosine. Tyrosine reduce phosphomolybdate compound to tungsten blue in alkaline condition, which has a absorption peak in 680 nm.

**Required but not provided:**

Spectrophotometer, water-bath, adjustable pipette, 1 mL glass cuvette, methylbenzene, distilled water and 50 meshes sieve (or smaller).

**Protocol:**

### I. Sample preparation

Fresh soil sample are dried by natural air or put into oven at 37°C, and then filter through a 30 ~ 50 meshes sieve.

### II. Protocol:

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

Reagent name	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Soil sample (g)	0.1	0.1	-	-
Reagent I (μL)	100	100	-	-

Reagent II (μL)	200	-	-	-
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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)	-	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 supernatant and detect the absorbance at 680 nm, 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 just need test once or twice. Each test tube should set one control tube.

### III. Calculation:

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

$$\text{Soil Alkaline Protease activity (U/g)} = C_S \times \Delta A_T \div \Delta A_S \times V_{RT} \div W \div T = 0.1 \times \Delta A_T \div \Delta A_S \div W$$

$C_S$ : Concentration of standard tube, 0.2 μmol/mL;

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

$T$ : Reaction time, 1 day=24 hours;

$W$ : Sample weight.

### 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. Take 0.1g of clover soil in 1.5 mL EP tube, as control tube and measuring tube, operate according to the determination steps, use 96 well plate to measure:  $\Delta A_T = A_T - A_C = 0.320 - 0.232 = 0.088$ ,  $\Delta A_S = A_S - A_B = 0.540 - 0.028 = 0.512$ , calculate the enzyme activity according to the soil mass: soil alkaline protease (U/g soil sample) =  $0.05 \times \Delta A_T \div \Delta A_S \div W = 0.1 \times 0.088 \div 0.512 \div 0.1 = 0.1718$  U/g soil sample.

2. Take 0.1g of forest 10 soil sample in 1.5 mL EP tube, as control tube and test tube respectively, operate according to the determination steps, use 96 well plate to measure  $\Delta A_T = A_T - A_C = 0.553 - 0.460 = 0.093$ ,  $\Delta A_S = A_S - A_B = 0.540 - 0.028 = 0.512$ , calculate the enzyme activity according to the

soil quality:

soil alkaline protease (U/g soil sample) =  $0.05 \times \Delta A_{T \div A_S} \div W = 0.1 \times 0.093 \div 0.512 \div 0.1 = 0.1816$  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:

BC0270/BC0275 Soil Neutral Protease Activity Assay Kit

BC0860/BC0865 Soil Acid Protease Activity Assay Kit