

# Soil Alkaline Protease Activity Assay Kit

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

**Detection equipment:** Spectrophotometer/Microplate reader

**Cat No:** NA0374

**Size:** 100T/48S

## Components:

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

**Reagent II:** Powder×1, store at 4°C; add 10 mL of Reagent I before use, mix thoroughly, and stir in boiling water and dissolve;

**Reagent III:** Powder×1, store at 4°C; add 10 mL of distilled water before use, mix thoroughly;

**Reagent IV:** Liquid 20 mL×1, store at 4°C;

**Reagent V:** Liquid 5 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 phosphomolybdic compound to tungsten blue in alkaline condition, which has an absorption peak in 680 nm.

## Required but not provided:

Spectrophotometer/Microplate reader, water-bath, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, methylbenzene, distilled water and 30 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/microplate reader for 30 min, 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. Test

Reagent name	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Soil sample (g)	0.05	0.05	-	-
Reagent I (μL)	50	50	-	-
Reagent II (μL)	100		-	-
Mix thoroughly and then reaction for 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)	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°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; each test tube should set a 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).

$$\text{Soil Alkaline Protease activity (U/g)} = C_S \times \Delta A_T \div \Delta A_S \times V_{RT} \div W \div T = 0.05 \times \Delta A_T \div \Delta A_S \div W$$

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

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

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

$W$ : Sample weight, g.

### Note:

When the absorbance value is more than 1.5, 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 0.05g 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:  $\Delta A_T = A_T - A_C = 0.234 - 0.180 = 0.054$ ,  $\Delta A_S = A_S - A_B = 0.374 - 0.045 = 0.329$ , calculate the enzyme activity according to the soil mass: soil alkaline protease (U/g soil sample) =  $0.05 \times \Delta A_T \div \Delta A_S \div W = 0.05 \times 0.054 \div 0.329 \div 0.05 = 0.1641$  U/g soil sample.

2. Take 0.05g 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  $\Delta A_T = A_T - A_C = 0.829 - 0.566 = 0.263$ ,  $\Delta A_S = A_S - A_B = 0.374 - 0.045 = 0.329$ , calculate the enzyme activity according to the soil quality: soil alkaline protease (U/g soil sample) =  $0.05 \times \Delta A_T \div A_S \div W = 0.05 \times 0.263 \div 0.329 \div 0.05 = 0.7994$  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:**

NA0847/NA0605 Soil Neutral Protease Activity Assay Kit

NA0805/NA0563 Soil Acid Protease Activity Assay Kit