

# Soil Leucine Aminopeptidase (S-LAP) Activity Assay Kit

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

**Operation Equipment:** Microplate reader/Spectrophotometer

**Catalog Number:** BC4025

**Size:**100T/48S

## Components:

Reagent I: 30 mL×1, stored at 4°C.

Reagent II: Powder×1. storage at 4°C and protected from light. Before use, add 3 mL of **acetone** (self-provided reagent) into the bottle, fully dissolve it.

## Product Description

S-LAP is a kind of enzyme that can hydrolyzes the N-terminal of peptide chain to leucine, which is secreted by soil microorganism. The changes of S-LAP activity are closely related to some pathological states.

S-LAP decomposes L-leucine-p-nitroaniline to p-nitroaniline, the latter has the maximum absorption peak at 405nm, and the activity of S-LAP is calculated by measuring the high rate of absorption value.

## Reagents and Equipment Required but Not Provided.

Scales, centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, **toluene**, **acetone**, 30 mesh sieve (or smaller).

## Procedure

### I. Sample processing:

The fresh soil samples are dried naturally and screened with 30-50 mesh.

### II. Determination steps:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 405 nm, set zero with the distilled water.
2. Add reagents in turn according to the following table:

Reagent name	Test tube(T)	Contrast tube(C)
Soil sample (g)	0.03	0.03
Toluene (μL)	15	15
Shake and mix well, and let stand for 15 minutes at room temperature.		
Reagent I (μL)	255	255
Reagent II (μL)	30	-
After reaction in water bath at 30°C for 1 hour, boil immediately for 5 minutes. Water cooling to room temperature.		
Reagent II (μL)	-	30

Centrifugate at  $14000 \times g$  for 10 minutes at room temperature, take 200  $\mu\text{L}$  of supernatant and measure the absorbance value at 405 nm, record it as  $A_T$  and  $A_C$  respectively, calculate  $\Delta A = A_T - A_C$ .

### III. Calculate activity of S-LAP

(1) Calculated by micro glass cuvette

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of p-nitrophenol per day every gram of soil sample.

$$\text{S-LAP (U/g)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div W \div T = 0.507 \times \Delta A \div W$$

$\epsilon$ : Molar extinction coefficient of p-nitroaniline:  $9.87 \times 10^3 \text{ L/mol/cm}$ ;

$d$ : Light diameter of cuvette, 1 cm;

$V_{RT}$ : The total volume of reaction,  $300 \mu\text{L} = 3 \times 10^{-4} \text{ L}$ ;

$W$ : Mass of soil sample, g;

$T$ : Reaction time, 60 minutes;

$10^9$ : Unit conversion coefficient,  $1 \text{ mol} = 10^9 \text{ nmol}$ .

(2) Calculated by 96 well plate

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of p-nitrophenol per day every gram of soil sample.

$$\text{S-LAP (U/g)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div W \div T = 0.844 \times \Delta A \div W$$

$\epsilon$ : Molar extinction coefficient of p-nitroaniline:  $9.87 \times 10^3 \text{ L/mol/cm}$ ;

$d$ : Light diameter of cuvette, 0.6 cm;

$V_{RT}$ : The total volume of reaction,  $300 \mu\text{L} = 3 \times 10^{-4} \text{ L}$ ;

$W$ : Mass of soil sample, g;

$T$ : Reaction time, 60 minutes;

$10^9$ : Unit conversion coefficient,  $1 \text{ mol} = 10^9 \text{ nmol}$ .

#### Experimental Examples:

1. Take two tubes of 0.03g clover soil samples and record them as the measuring tube and the control tube respectively. Follow the measurement steps using 96-well plate to calculate to calculate  $\Delta A = A_t - A_c = 0.6 - 0.17 = 0.43$ , and calculate the enzyme activity:

$$\text{S-LAP activity (U/g soil)} = 0.507 \times \Delta A \div W = 0.507 \times 0.43 \div 0.03 = 7.267 \text{ U/g soil.}$$

2. Take two tubes of 0.03g soil sample and record them as the measuring tube and the control tube respectively. Follow the measurement steps using 96-well plate to calculate  $\Delta A = A_t - A_c = 0.569 - 0.128 = 0.441$ , and calculate the enzyme activity:

$$\text{S-LAP activity (U/g soil)} = 0.507 \times \Delta A \div W = 0.507 \times 0.441 \div 0.03 = 7.4529 \text{ U/g U/g soil}$$

#### Related Products:

BC0880/BC0885 Soil Alkaline Protease Activity Assay Kit

BC4010/BC4015 Soil  $\beta$ -Xylosidase(S- $\beta$ -XYS) Activity Assay Kit

BC3080/BC3085 Soil  $\alpha$ -glucosidase(S- $\alpha$ -GC) Activity Assay Kit

BC0240/BC0245 Soil Saccharase(S-SC) Activity Assay Kit