

# 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:** NA0362

**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 ×g for 10 minutes at room temperature, take 200 μL of supernatant and measure the absorbance value at 405 nm, record it as A<sub>T</sub> and A<sub>C</sub> respectively, calculate ΔA = A<sub>T</sub> - A<sub>C</sub>.

### 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.

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

ε: Molar extinction coefficient of p-nitroaniline: 9.87×10<sup>3</sup> L/mol/cm;

d: Light diameter of cuvette, 1 cm;

V<sub>RT</sub>: The total volume of reaction, 300 μL = 3×10<sup>-4</sup> L;

W: Mass of soil sample, g;

T: Reaction time, 60 minutes;

10<sup>9</sup>: Unit conversion coefficient, 1mol = 10<sup>9</sup> 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.

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

ε: Molar extinction coefficient of p-nitroaniline: 9.87×10<sup>3</sup> L/mol/cm;

d: Light diameter of cuvette, 0.6 cm;

V<sub>RT</sub>: The total volume of reaction, 300 μL = 3×10<sup>-4</sup> L;

W: Mass of soil sample, g;

T: Reaction time, 60 minutes;

10<sup>9</sup>: Unit conversion coefficient, 1mol = 10<sup>9</sup> 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 ΔA=A<sub>t</sub>-A<sub>c</sub>=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 ΔA=A<sub>t</sub>-A<sub>c</sub>=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:

NA0804/NA0374 Soil Alkaline Protease Activity Assay Kit

NA0364/NA0363 Soil β-Xylosidase(S-β-XYS) Activity Assay Kit

NA0645/NA0404 Soil α-glucosidase(S-α-GC) Activity Assay Kit

NA0850/NA0608 Soil Saccharase(S-SC) Activity Assay Kit