

**Soil Leucine Aminopeptidase (S-LAP) Activity Assay Kit - Microplate Method****Product Information**

**Product code:** 67055

S-LAP is a class of enzymes secreted by soil microorganisms that can hydrolyze peptide chains with N-terminal leucine. Changes in S-LAP activity are closely related to certain pathological states of the body.

S-LAP decomposes L-leucyl-p-nitroanilide to produce p-nitroaniline, which has a maximum absorption peak at 405 nm. S-LAP activity can be calculated by measuring absorbance at 405 nm.

**Package Contents**

Size	Code	Item	Quantity
100T	67055.1	Reagent I	1 bottle
100T	67055.2	Reagent II	1 bottle
100T	67055.m	Instructions	1 copy

**Quality Standards and Safety Information**

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--

**Transportation and Storage**

Transportation	Transport with ice packs.
Storage	Store Reagent I at 4 °C. Store Reagent II at -20 °C, protected from light. Shelf life is 180 days.

**Instructions for Use****1. Reagent Preparation**

Add 15 mL Reagent I to the Reagent II bottle and dissolve thoroughly. If dissolution is difficult, heat in a 60 °C water bath for about 30 min to promote dissolution.

Incubate in a water bath at 37 °C for mammals or 25 °C for other species for 10 min or longer.

Aliquot unused reagent and store at -20 °C. Do not freeze and thaw repeatedly.

**2. Operating Procedure**

1. Preheat the microplate reader for 30 min or longer, set the wavelength to 405 nm, and zero with distilled water.
2. Add samples according to the table below.

Component	Control Tube	Assay Tube
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Component	Control Tube	Assay Tube
Fresh soil sample (g)	0.05	0.05
Reagent I ( $\mu\text{L}$ )	300	-
Reagent II ( $\mu\text{L}$ )	-	300

- Mix well and react with shaking at 37 °C for 1 h.
- Centrifuge at 8000g, 4 °C for 10 min.
- Transfer 200  $\mu\text{L}$  of supernatant into a 96-well plate and measure the absorbance at 405 nm as A.
- Calculate  $\Delta A = A_{\text{assay}} - A_{\text{control}}$ . Set one control tube for each assay tube.

### 3. S-LAP Activity Calculation

Unit definition: the generation of 1  $\mu\text{mol}$  p-nitroaniline per day by 1 g soil sample is defined as one unit of enzyme activity.

$$\text{S-LAP } (\mu\text{mol/d}\cdot\text{g}^{-1}, \text{ soil sample}) = \Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^6 \div W \div T = 29.6 \times \Delta A$$

$V_{\text{total reaction}}$	Total volume of the reaction system, $3 \times 10^{-4}\text{L}$
$\epsilon$	Molar extinction coefficient of p-nitroaniline, $9.72 \times 10^3\text{L/mol}\cdot\text{cm}^{-1}$
$d$	Optical path length of the cuvette, 0.5 cm
$T$	Reaction time, 1 h = 1/24 d
$W$	Sample mass, 0.05 g

### Precautions

- This 100T kit can test 48 samples.