EZMeta™ Soil Leucine Arylamidase (S-LAP) Colorimetric Activity Kit

Cat #: D-AKC4020

Size: 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Soil

Assay Principle

Soil Leucine Arylamidase (LAP) can hydrolyze enzyme with N-terminal leucine at the peptide chain, and was secreted by soil microorganism. The S-LAP activity changes are closely related to certain pathological states of the body. EZMeta™ Soil Leucine Arylamidase (S-LAP) Colorimetric Activity Kit provides a simple, convenient and rapid Soil LAP activity detection method. The detection principle is that the p-nitroaniline produced by S-LAP hydrolysis of L-leucyl-p-nitroaniline, there is a characteristic absorption peak at 405 nm. The S-LAP activity is calculated by measuring of absorbance changes.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
Reagent Ⅰ	70 mL	4°C
Reagent Ⅱ	1	4°C, protected from light

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 405 nm

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·Centrifuge, water bath

·Deionized water

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent Ⅱ: For 96 T, add 30 mL Reagent I before use; Fully dissolve it for use (when it is difficult to dissolve, it can be heated in a 60°C water bath for 30 min to promote dissolution), aliquoted and stored at -20°C. Avoid repeated freezing and thawing.

Sample Preparation

Fresh soil was recommended

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 405 nm, visible spectrophotometer was returned to zero with deionized water.

2. Enzymatic reaction (the following operations are performed in a 1.5 mL centrifuge tube).

Reagent	Test Tube (μL)	Control Tube (μL)
Sample (mg)	50	50
Reagent Ⅰ	0	300
Reagent Ⅱ	300	0

Mix well, put it in a water bath at 37°C shaking for 1 h, centrifuge at 8,000 g at 4°C for 10 min, and take 200 μL supernatant and add into 96-well plate or microglass cuvette. The absorbance value was determined by 405 nm, which was recorded as A_{Test} and $A_{Control}$, calculated $\Delta A_{Test}=A_{Test}-A_{Control}$, One control tube was suggested for each test.

Note: In order to guarantee the accuracy of experimental results, pre-experiments were suggested using 2-3 samples with potential significant differences. Increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula

Definition of unit: 1 nmol of p-nitroaniline produced per gram soil per minute is defined as an enzyme activity unit.

Calculated S-LAP activity using microcuvette:

S-LAP (U/g sample) = $\Delta A \times V_{Total\ reaction} \div (\varepsilon \times d) \times 10^9 \div W \div T =$ **$1.013 \times \Delta A \div W$**

$V_{Total\ reaction}$: Total volume of reaction system, 3×10^{-4} L; ε: molar extinction coefficients of p-nitroaniline, 9.87×10^3 L/mol/cm; d: 96-well plate optical diameter, 0.5 cm; T: Reaction time, 1 h=60 min; W: Sample weight, g; 10^9 : coefficients, 1 mol=1×10^9 nmol.

B. Microglass cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Typical Data

Example 1: Take 0.05 g fresh soil and S-LAP activity was detected in 96-well plate, the resulting $\Delta A_{Test} = A_{Test} - A_{Control}$ = 0.266-0.053=0.213, the S-LAP activity in this soil sample (U/g) =1.013×0.213÷0.05=4.32 U/g.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.