EZMeta™ Leucine Arylamidase (LAP) Colorimetric Activity Kit

Cat #: D-AKC4021

Size: 48T / 96T

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

Product Information

Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum

Assay Principle

Leucine Arylamidase (LAP) is a kind of enzyme that can hydrolyze the peptide with N-terminal leucine. It widely exists in liver, kidney, pancreas and other tissues, especially in liver. LAP decomposes L-Leucine-p-nitroanilide to generate p-nitroaniline, and the latter has a maximum absorption peak at 405 nm. LAP activity is calculated by measuring the rate of absorbance increase.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	57.5 mL	115 mL	4°C
Reagent II	1	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, ultrasonic crusher

·Deionized water, ice

·Homogenizer or mortar

Reagent Preparation

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

Reagent II: For 48 T, add 7.5 mL Reagent I before use; For 96 T, add 15 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 about 30 min to promote dissolution), and store it separately at -20°C. Repeated freezing and thawing are prohibited.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C for one month.

1.Animal and plant tissue: Weigh 0.1 g tissue, add 1 mL Reagent I and homogenize on ice (Tissue mass (g): Reagent I volume (mL) is 1:10). Centrifuge at 10,000 g for 10 min at 4°C. Take the supernatant and put it on ice for testing.

2.Cells or bacteria: Collect 5×10^6 cells or bacteria and wash cells or bacteria with pre-cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent I to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

3.Serum (plasma): Test directly.

Assay Procedure

1. Preheat the microplate reader or visible 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 operated in the 96-well plate or microglass cuvette)

Reagent	Test Tube (μL)	Blank Tube (μL)
Supernatant	50	0
Reagent I	0	50
Reagent II	150	150

After thorough mixing, the absorbance value A_1 at 405 nm for 30 s was measured, and it was quickly placed in a 37°C water bath for 3 min (The microplate reader with temperature control function can adjust the temperature to 37°C), take out the absorbance A_2 at 210 s after rapid drying, and calculate $\Delta A=A_2-A_1$.

Note: Blank well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Reagent I, 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.

Calculation of LAP activity unit:

A. 96-well UV plates calculation formula as below

1. Calculation of LAP activity in serum (plasma)

Definition of unit: 1 nmol of p-nitroaniline generated per min per mL serum (plasma) is defined as a unit of enzyme activity.

$LAP(U/mL)=[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div V_{Sample}\div T=\mathbf{274.35\times\Delta A}$

2. Calculation of LAP activity in tissues, bacteria or cells

(1) Calculated by protein concentration

Definition of unit: 1 nmol of p-nitroaniline generated per min per mg tissue protein is defined as a unit of enzyme activity.

$LAP(U/mg\ prot)=[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T=\mathbf{274.35\times\Delta A\div Cpr}$

(2) Calculated by sample fresh weight

Definition of unit: 1 nmol of p-nitroaniline generated per min per g tissue is defined as a unit of enzyme activity.

LAP(U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(W×V_{Sample}÷$V_{Total\ Sample}$)÷T=**274.35×ΔA÷W**

(3) Calculated by bacteria or cell number

Definition of unit: 1 nmol of p-nitroaniline generated per min per 10^4 bacteria or cells is defined as a unit of enzyme activity.

LAP(U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(500×V_{Sample}÷$V_{Total\ Sample}$)÷T=**0.548×ΔA**

V_{Total}: total reaction volume, 2×10^{-4} L; ε: LAP molar extinction coefficien, 9.72×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.05 mL; $V_{Total\ Sample}$: Reagent I volume added, 1 mL; T: reaction time, 3 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g; 500: Total number of bacteria or cells, 5×10^6.

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

Experimental example:

The mouse serum was tested in 96-well plate directly according to the measurement steps, ΔA=1.013-0.867=0.145, calculated enzyme activity:

LAP(U/mL)=274.35×ΔA=274.35×0.145=39.78 U/mL

Disclaimer

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