Leucine Aminopeptidase（LAP）Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4145

Size： 100T/96S

Components:

Reagent I: 120 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃ and protect from light. Add 2.5 mL of acetone (**self-provided reagent**) to dissolve before use.

Product Description:

Leucine aminopeptidase (LAP) is a kind of membrane binding enzyme, which widely exists in liver, gallbladder, pancreas and other tissues. LAP participates in the degradation and renewal of tissue proteins and some peptides. Because of the damage of liver cells, the activity of serum LAP in patients with various liver diseases has increased in varying degrees. LAP can be used as a preliminary detection index for various liver diseases, especially for the differential diagnosis of liver cancer.

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

Reagents and Equipment Required but Not Provided:

Scales, low temperature centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, acetone, homogenizer/mortar.

Procedure:

I. Sample preparation:

1. Tissue:

The mass of tissue (g): the volume of Reagent I (mL) = 1:5~10 (it is recommended to weigh about 0.1 g of tissue, add 1 mL of Reagent I. Homogenate in ice bath. Centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant and put it on ice for test.

2. Cells:

The number of cells (10^4): the volume of Reagent I (mL) is 500~1000:1 (it is recommended to add 1 mL of Reagent I to 5 million cells), the cells are broken by ice bath ultrasound (Power: 300 w, ultrasound: 3 s, interval: 7 s, total time: 3 min). Centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant and put it on ice for test.

3. Liquid:

Direct detection.

II. Determination procedure

(1) Preheat the spectrophotometer/microplate reader 30 minutes, adjust the wavelength to 405 nm

and set zero with distilled water.

(2) Add reagent table: add the following reagents to the micro glass cuvette/96 well plate respectively.

Reagent (μL)	Test tube (T)	Blank tube (B)
Reagent I	-	10
The supernatant of sample	10	-
Reagent I	170	170
Reagent II	20	20

Add the above reagents into the micro glass cuvette/96 well plate respectively, mix them well, measure the absorbance value A_1 at 405 nm for 30 s, quickly put them into a 37°C-water bath for 3 minutes (if there is a microplate reader with temperature control function, the temperature can be adjusted to 37°C). Take them out and dry them quickly, measure the absorbance value A_2 at 210 s, calculate the $\Delta A_T = A_{2T}-A_{1T}$, $\Delta A_B = A_{2B}-A_{1B}$, $\Delta A = \Delta A_T-\Delta A_B$. Blank tube only needs to be done once or twice.

III. Calculation formula of enzyme activity:

1. Calculated according to the micro glass cuvette:

(1) Calculation of liquid LAP activity:

Unit definition: One unit of enzyme activity is defined as the amount enzyme of that catalyzes the produce of 1 nmol of p-nitroaniline per minute every milliliter of blood.

$LAP\ (U/mL) = [\Delta A \times V_{RV} \div (\varepsilon \times d) \times 10^9] \div V_{SV} \div T = 675.4 \times \Delta A$

(2) Calculation of LAP activity in tissues, bacteria or cells:

a. calculation based on concentration of sample protein:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the produce of 1 nmol p-nitroaniline per minute per milligram tissue protein.

$LAP\ (U/mg\ prot) = [\Delta A \times V_{RV} \div (\varepsilon \times d) \times 10^9] \div (V_{SV} \times Cpr) \div T = 675.4 \times \Delta A \div Cpr$

b. calculation based on fresh weight of sample:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the produce of 1 nmol p-nitroaniline per minute per gram tissue weight.

$LAP\ (U/g\ fresh\ weight) = [\Delta A \times V_{RV} \div (\varepsilon \times d) \times 10^9] \div (W \times V_{SV} \div V_{STV}) \div T = 675.4 \times \Delta A \div W$

c. calculation based on cell density:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the produce of 1 nmol p-nitroaniline per minute per 10 thousand cells.

$LAP\ (U/10^4\ cells) = [\Delta A \times V_{EV} \div (\varepsilon \times d) \times 10^9] \div (500 \times V_{SV} \div V_{STV}) \div T = 1.35 \times \Delta A$

V_{RV}: Total volume of reaction system, 2×10^{-4} L;

ε: Molar extinction coefficient of p-nitroaniline, 9.87×10^3 L/mol/cm;

10^9: Unit conversion coefficient, 1 mol = 10^9 nmol;

d: Light diameter of cuvette, 1 cm;

V_{SV}: Added the volume of sample, 0.01 mL;

V_{STV}: Added the total volume of reagent, 1 mL;

T: Reaction time, 3 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: The numbers of cells or bacteria, 5 million cells.

2. Calculation according to 96 well plate:

Change the d-1cm in the above calculation formula to d-0.6cm (96 well plate optical diameter) for calculation.

Note;

1. When ΔA is greater than 0.5 or the value of A is greater than 1.5, it is recommended to dilute the supernatant of sample with reagent before determination.
2. The change of ΔA_B is less than 0.01.

Experimental Examples:

1. Take 0.1g of mouse kidney and add 1mL reagent to homogenize and grind, take the supernatant and operate according to the measurement steps, and calculate ΔAt = A2t-A1t=0.451-0.372=0.079, ΔAb = A2b-A1b=0.012-0.011=0.001, ΔA=ΔAt -ΔAb=0.079-0.001=0.078, the enzyme activity is calculated according to the sample quality:

LAP（U/g weight）=675.4×ΔA÷W=675.4×0.078÷0.1=526.812 U/g weight。

2. Take chicken serum directly and operate according to the determination procedure, and the calculated calculation ΔAt= A2t-A1t=0.165-0.148=0.017, ΔAb=A2b-A1b=0.012-0.011=0.001, ΔA=ΔAt-ΔAb=0.017-0.001=0.016, the enzyme activity is calculated according to the sample quality:

LAP（U/g weight）=675.4×ΔA=675.4×0.016=10.8064 U/g weight。

Related Products:

BC1550/BC1555 Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit

BC1560/BC1565 Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit

BC0290/BC0295 Proline(PRO) Content Assay Kit

Experimental Examples:

1. Take 0.1g of mouse kidney and add 1mL reagent to homogenize and grind, take the supernatant and operate according to the measurement steps, measure by the micro glass cuvette and calculate ΔAt = A2t-A1t=0.4537-0.3691=0.0846, ΔAb = A2b-A1b=0.0157-0.0133=0.0024, ΔA=ΔAt -ΔAb=0.0846-0.0024=0.0822, the enzyme activity is calculated according to the sample quality:

LAP（U/g weight）=675.4×ΔA÷W=675.4×0.0822÷0.1=555.1788 U/g weight。

2. Take chicken serum directly and operate according to the determination procedure, measure by the micro glass cuvette and calculate ΔAt= A2t-A1t=0.094-0.0798=0.0142, ΔAb=A2b-A1b=0.0157-0.0133=0.0024, ΔA=ΔAt-ΔAb=0.0142-0.0024=0.0118, the enzyme activity is calculated according to the sample quality:

LAP（U/g weight）=675.4×ΔA=675.4×0.0118=7.9697 U/g U/g weight。

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