Technical Manual

Leucine Aminopeptidase (LAP) Activity Assay Kit

- Catalogue Code: MAES0190
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

5.2-201.8 U/L

Sensitivity:

5.2 U/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Leucine aminopeptidase (LAP) is a kind of specific hydrolase ubiquitous in animal and plant tissues. It can hydrolyze not only leucine compounds, but also a variety of other amino-amides and polypeptides. When liver cancer, extrahepatic bile duct obstruction, intrahepatic cholestasis, or other intrahepatic space-occupying lesions, as well as liver parenchymal cell damage, LAP enzyme activity increases, and especially in the early stage of liver cancer, the enzyme activity increases sharply. Therefore, the detection of LAP enzyme activity is of certain clinical significance for the diagnosis and treatment of hepatobiliary diseases.

3. Intended Use

This kit can be used to measure leucine aminopeptidase (LAP) activity in animal tissue, serum, plasma and other liquid samples.

4. Detection Principle

LAP can catalyze the substrate L-leucine-4-nitroaniline to produce p-nitroaniline, which has the maximum absorption peak at the wavelength of 405 nm. The enzyme activity of LAP can be calculated by measuring the increasing OD value of the system.

5. Kit Components & Storage

Item	Specification	Storage
Extracting Solution	60 mL × 2 vials	2-8°C, 6 months
Substrate	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
p-Nitroaniline Standard	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath
- Incubator
- Centrifuge
- Microplate Reader (405 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Acetone

6. Assay Notes:

Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate working solution:** Dissolve a vial of substrate powder with 1.2 mL acetone (self-prepared). Prepare the fresh solution before use and preserve it on ice for detection.

3. Preparation of **p-nitroaniline standard stock solution (50 mmol/L):** Dissolve a vial of standard powder with 1 mL acetone (self-prepared) fully.

4. Preparation of **p-nitroaniline standard solution (1 mmol/L):** Dilute 50 mmol/L p-nitroaniline standard stock solution with acetone (self-prepared) at a ratio of 1:49. Prepare the fresh solution before use and preserve it on ice for detection.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of extracting solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (5.2-201.8 U/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
Human serum	1
Dog serum	1
Rat serum	1
Cynomolgus monkey serum	1
10% Rat spleen tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat lung tissue homogenate	1

Note: The diluent is extracting solution.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 450 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 1 mmol/L p-nitroaniline standard solution with extracting solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1.0 mmol/L.

The measurement of samples

1. **Standard well:** add 180 μL of extracting solution into the corresponding standard wells.
   Sample well: take 10 μL of sample into the corresponding sample wells, and add 170 μL of extracting solution.

2. **Standard well:** take 20 μL of standard with different concentrations into the corresponding wells.
   Sample well: add 20 μL of substrate working solution into the corresponding wells.

3. Mix fully for 5 s with microplate reader, measure the OD values of each well at 405 nm with microplate reader, recorded as A_1, and then incubate accurately at 37°C for 10 min, measure the OD values of each well at 405 nm with microplate reader, recorded as A_2, $\triangle A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of $A_{2(standard)}$).

Operation Table

	Standard well	Sample well
Sample (μL)		10
Extracting solution (μL)	180	170
Standards with different concentrations (μL)	20	
Substrate working solution (μL)		20
Mix fully for 5 s with microplate reader, measure the OD values of each well at 405 nm with microplate reader, recorded as A_1, and then incubate accurately at 37°C for 10 min, measure the OD values of each well at 405 nm with microplate reader, recorded as A_2, $\triangle A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of $A_{2(standard)}$)		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

Definition: the amount of enzyme in 1 L of serum (plasma) that catalyze the substrate to produce 1 μmol p-nitroaniline at 37°C for 1 min is defined as 1 unit.

$$\text{LAP activity (U/L)} = \frac{\Delta A - b}{a} \times V_1 \div V_2 \div T \times 1000^* \times f$$

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve.
ΔA: $\triangle A = A_2 - A_1$.
V_1: The volume of added substrate working solution, 20 μL;
V_2: The volume of added sample, 10 μL;
1000*: 1 mmol = 1000 μmol
T: Reaction time (10 min).
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

2. Tissue sample:

Definition: the amount of enzyme in 1 g of tissue protein that catalyze the substrate to produce 1 μmol p-nitroaniline at 37°C for 1 min is defined as 1 unit.

$$\text{LAP activity (U/gprot)} = \frac{\Delta A - b}{a} \times V_1 \div V_2 \div T \times 1000^* \times f \div C_{pr}$$

12. Performance Characteristics

Detection Range	5.2-201.8 U/L
Sensitivity	5.2 U/L
Average inter-assay CV (%)	6.4
Average intra-assay CV (%)	4.1

Analysis

For human serum, take 10 μL of human serum to corresponding wells and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.4501 x – 0.0181, the initial average OD value of the sample is 0.214, recorded as A_1, the average OD value of the sample after incubation for 10 min is 0.240, recorded as A_2, $\Delta A = A_2 - A_1 = 0.026$, and the calculation result is:

$$\textbf{LAP activity (U/L)} = (0.026 + 0.0181) \div 0.4501 \times 20 \div 10 \div 10 \times 1000$$

$$= 19.60 \text{ U/L}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Please touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assaygenie.com