（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K568-M

Specification：96T(80 samples)

Measuring instrument：Microplate reader（405 nm）

Detection range：5.2-201.8 U/L

Elabscience® Leucine Aminopeptidase (LAP) Activity Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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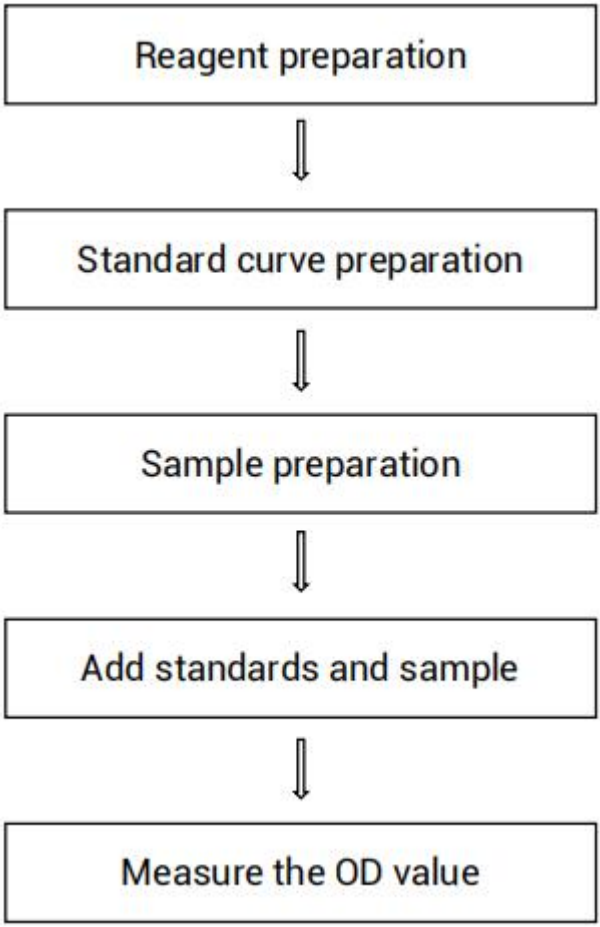
Assay summary



Intended use

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

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.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Substrate	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 3	p-Nitroaniline Standard	Powder × 1 vial	2-8°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (405 nm), Micropipettor, Water bath, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Anhydrous ethanol

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution：

Dissolve one vial of substrate with 1.2 mL of anhydrous ethanol (self-prepared), mix well to dissolve. Keep it on ice during use. Store at 2-8°C for 7 days protected from light.

③ The preparation of 50 mmol/L p-nitroaniline standard stock solution：

Dissolve one vial of p-nitroaniline standard with 1 mL of anhydrous ethanol (self-prepared), mix well to dissolve.

④ The preparation of 1 mmol/L p-nitroaniline standard solution：

Add 20 μL of 50 mmol/L p-nitroaniline standard stock solution and 980 μL of extracting solution, mix well. The standard solution should be prepared on spot and keep it on ice during use. Store at 2-8°C for 7 days protected from light.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with extracting solution diluent 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1.0**
1 mmol/L standard (μL)	0	40	60	80	120	160	180	200
Extracting solution (μL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation:

Serum (plasma): detect directly.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

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

Operating steps

① Standard well: add 180 μL of extracting solution into the corresponding standard wells.

Sample well: add 10 μL of sample into the corresponding sample wells, and add 170 μL of extracting solution.

② Standard well: add 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 $A_{2\,(standard)}$).

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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.

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

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.

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

[Note]

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

f: Dilution factor of sample before test;

T: Reaction time: 10 min;

C_{pr}: The concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.5	65.8	175.0
%CV	4.5	4.0	3.8

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.5	65.8	175.0
%CV	6.2	6.0	7.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.25	0.55	0.82
Observed Conc. (mmol/L)	0.3	0.5	0.8
Recovery rate (%)	101	99	97

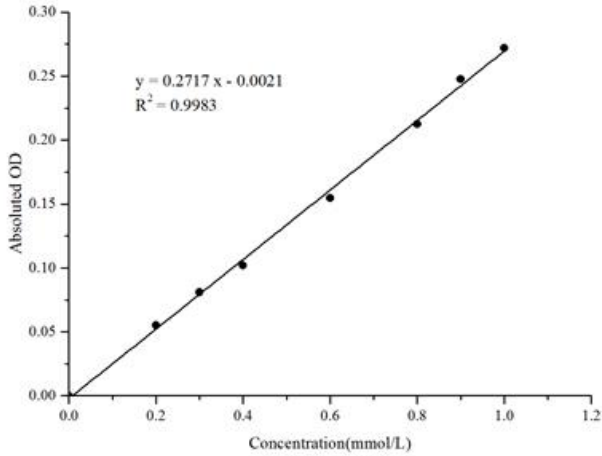
Sensitivity

The analytical sensitivity of the assay is 5.2 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (mmol/L)	**0.0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1.0**
Average OD	0.056	0.111	0.137	0.158	0.211	0.269	0.304	0.328
Absoluted OD	0.000	0.055	0.081	0.102	0.155	0.213	0.248	0.272



Appendix Π Example Analysis

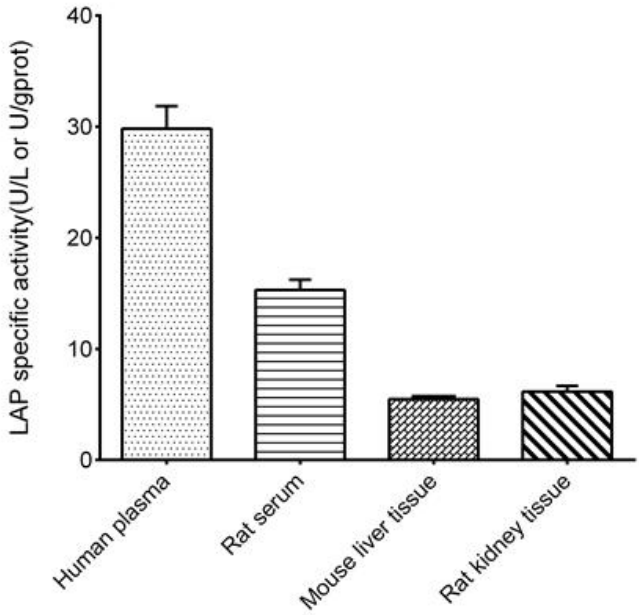
Example analysis：

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:

LAP activity (U/L) = (0.026 + 0.0181) ÷ 0.4501 ×20 ÷10 ÷ 10 ×1000 = 19.60 U/L

Detect human plasma, rat serum, 10% rat liver tissue homogenate (the concentration of protein is 7.31 gprot/L) and 10% rat kidney tissue homogenate (the concentration of protein is 4.52 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.