Product Information & Manual

Leucine Aminopeptidase (LAP) Activity Assay Kit (Colorimetric)
NBP3-24514

Contact

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Novus kits are guaranteed for 6 months from date of receipt.

For research use only. Not for diagnostic or therapeutic procedures.

Leucine Aminopeptidase (LAP) Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24514

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 5.2 U/L

Detection range: 5.2-201.8 U/L

Average intra-assay CV (%): 4.1

Average inter-assay CV (%): 6.4

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Substrate	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 3	p-Nitroaniline Standard	Powder × 1 vial	2-8℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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.			

▲Materials prepared by users

Instruments

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

Reagents

Double distilled water, Acetone

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ The key point of the assay

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

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 working solution:
   Dissolve a vial of powder with 1.2 mL acetone (self-prepared). Prepare the fresh solution before use and preserve it on ice for detection.
2. Preparation of 50 mmol/L p-nitroaniline standard stock solution:
   Dissolve a vial of standard powder with 1 mL acetone (self-prepared) fully.
3. Preparation of 1 mmol/L p-nitroaniline standard solution:
   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.

▲Sample preparation

1. Serum (Plasma): Detect directly.

2. Tissue: Accurately weigh the tissue sample, add 9 times the volume of reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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 reagent 1.

Assay protocol

▲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.

▲Detailed operating steps

The preparation of standard curve

Dilute 1 mmol/L p-nitroaniline standard solution with reagent 1 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:

Number	Standard concentrations (mmol/L)	1 mmol/L Standard(μL)	Reagen1 (μL)
A	0	0	200
B	0.2	40	160
C	0.3	60	140
D	0.4	80	120
E	0.6	120	80
F	0.8	160	40
G	0.9	180	20
H	1.0	200	0

The measurement of samples

1) Standard well: add 180 μL of reagent 1 into the corresponding standard wells.
   Sample well: take 10 μL of sample into the corresponding sample wells, and add 170 μL of reagent 1.
2) Standard well: take 20 μL of standard with different concentrations into the corresponding wells.
   Sample well: add 20 μL of reagent 2 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℃ for 10 min, measure the OD values of each well at 405 nm with microplate reader, recorded as A_2, △ $A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of $A_{2(standard)}$.

▲Summary operation table

	Standard well	Sample well
Sample (μL)		10
Reagent 1 (μL)	180	170
Standards with different concentrations (μL)	20	
Reagent 2 working solution (μL)		20
Mix fully, measure the OD values at 405 nm, recorded as A_1, and then incubate accurately at 37℃ for 10 min, measure the OD values at 405 nm, recorded as A_2, △ $A = A_2 - A_1$.		

▲Calculation

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 ℃ for 1 min is defined as 1 unit.

LAP activity (U/L) = (ΔA - b) ÷ a ×V_1 ÷V_2 ÷ T ×1000*× 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 ℃ for 1 min is defined as 1 unit.

LAP activity (U/gprot) = (ΔA - b) ÷ a ×V_1 ÷V_2 ÷ T ×1000*× f ÷ C_{pr}

Note:

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 reagent 2 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 Data

▲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