

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

Catalog No: E-BC-K1407-M

Specification: 48T (30 samples)/96T (78 samples)

Measuring instrument: Microplate reader (405-415 nm)

Detection range: 1.73-81.46 U/L

Elabscience® Lipoprotein Lipase (LPL) Activity Colorimetric 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

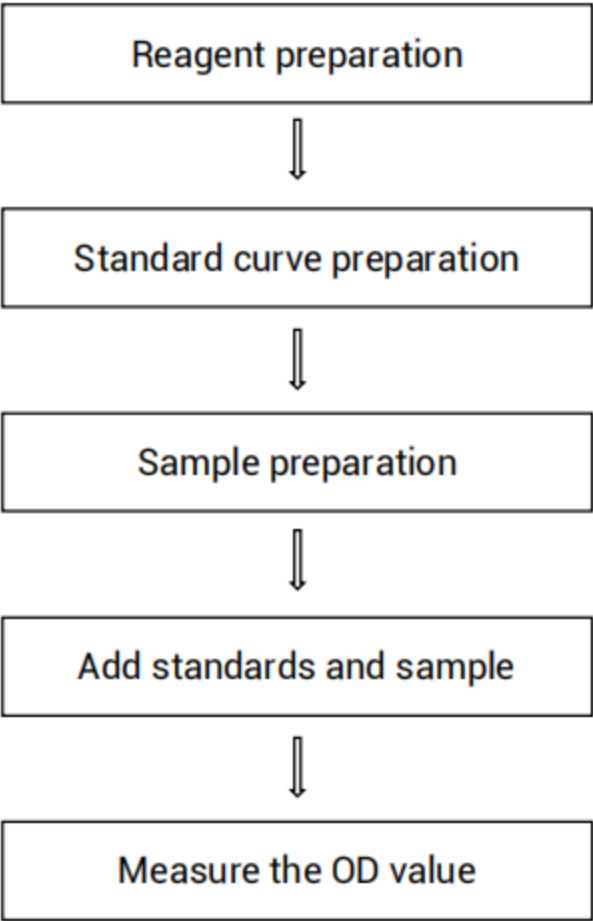
Website: www.elabscience.com

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 measure lipoprotein lipase (LPL) activity in serum and animal tissue samples.

Detection principle

Lipoprotein lipase is a core molecule located at the interface between blood circulation and tissue energy metabolism. By hydrolyzing triglycerides, it precisely allocates the lipid energy from dietary and hepatic sources to storage (adipose) or consumption (muscle) tissues. The fine regulation of its activity is essential for maintaining systemic lipid homeostasis.

The detection principle of this kit: lipoprotein lipase catalyzes the hydrolysis of the substrate to generate the colored product p-nitrophenol, which has a maximum absorption peak at 410 nm.

When this kit is used to detect tissue samples, the total protein concentration needs to be determined. It is recommended to use the BCA kit of our company (Catalog No : E-BC-K318-M) for the determination.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	42 mL×1 vial	42 mL×2 vials	-20°C, 12 months, shading light
Reagent 2	Buffer Solution	9 mL×1 vial	18 mL×1 vial	-20°C, 12 months, shading light
Reagent 3	Substrate	0.06 mL×1 vial	0.12 mL×1 vial	-20°C, 12 months, shading light
Reagent 4	10 mmol/L Standard Solution	1 mL×1 vial	1 mL×1 vial	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplatereader (405-415 nm, optimum wavelength: 410 nm), Incubator (37°C)

Materials required but not provided

Double distilled water, Dimethyl sulfoxide (DMSO)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Substrate Working Solution preparation :

Before testing, prepare a sufficient amount of Substrate Working Solution according to the test wells. For example, prepare 1000 μL of a Substrate Working Solution (mix 100 μL of Substrate and 900 μL of DMSO thoroughly). Stable for 8 h protected from light.

③ Preparation of 2 mmol/L Standard :

Before testing, prepare a sufficient amount of 2 mmol/L standard according to the test wells. For example, prepare 1000 μL of a 2 mmol/L standard solution (mix 800 μL of Buffer Solution and 200 μL of a 10 mmol/L standard thoroughly). The 2 mmol/L standard should be stable for 8 h.

④ The preparation of standard curve:

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

Dilute 2 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1, 1.4, 2 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	0	0.2	0.4	0.6	0.8	1	1.4	2
2 mmol/L Standard (μL)	0	20	40	60	80	100	140	200
Buffer Solution (μL)	200	180	160	140	120	100	60	0

Sample preparation

Serum: Direct detection. (For the relatively turbid serum samples, centrifuge at $10000 \times g$ for 5 min at 4°C and take the supernatant for detection.)

Tissue sample:

- 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 Extraction Solution with a dounce homogenizer at 4°C .
- ④ Centrifuge at $10000 \times g$ for 10 min at 4°C 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). (It is normal for the samples with high protein concentration to be slightly turbid; mix well before detection.)

Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2-3
10% Mouse kidney tissue homogenate	2-3
10% Mouse heart tissue homogenate	2-3
10% Mouse spleen tissue homogenate	2-3
10% Mouse lung tissue homogenate	2-3
Rats serum	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

- ① The samples should be thoroughly mixed, and bubbles should be avoided when adding.
- ② Reagent 3 should be tightly sealed in time after use, and stored at low temperature away from light.

Operating steps

Enzymatic reaction

- ① Blank well: Add 90 μL of buffer solution to the corresponding wells. (just need to conduct 1-2 tests).

Standard well: Add 90 μL of buffer solution to the corresponding wells.

Sample well: Add 80 μL of buffer solution to the corresponding wells.

- ② Add 10 μL of the test sample to the sample wells.
- ③ Add 10 μL of standard solution with different concentrations to the corresponding wells.

Add 10 μL of substrate working solution to the blank and sample wells.

- ④ Mix fully with microplate reader for 30 s. Measure the initial OD value (A_1) of each well at 410 nm with microplate reader.
- ⑤ Then incubate at 25°C for 5 minutes. Measure the OD value of each well at 410 nm using a microplate reader and record as A_2 .

(the standard curve is calculated using the A_2 value). (It is recommended to perform the measurement according to the notes below.)

Notes:

1. After adding Reagent 3 working solution, it is recommended to record the OD value once per minute for 5 consecutive minutes, and observe whether the OD value shows a rising trend at a constant speed within 5 min. For calculation, the initial OD value of the reaction is taken as A_1 , and the OD

value measured at 5 min is taken as A_2 .

2. If the sample activity is high, the sample concentration can be appropriately reduced, so that the reaction OD value changes linearly at a constant speed within 0-5 min, avoiding the excessive change of the blank value caused by the too long reaction time, which affects the result calculation. If the LPL activity of the sample is low, the measurement time can be extended to 10-20 min, and the OD value is read every 1-2 min. The interval where the data shows a linear relationship in the early and middle stages is selected for analysis and calculation, and the blank wells are also calculated synchronously with the measured values of the corresponding time period.

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 absolute OD value.
3. Plot the standard curve by using absolute 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:

Tissue sample:

Definition: At 25°C, the amount of enzyme required to catalyze the generation of 1 μmol of p-nitrophenol per minute per gram of tissue protein in the process of catalyzing the substrate is defined as one enzyme activity unit.

$$\text{LPL activity (U/gprot)} = (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) - b \div a \div T \div \text{Cpr} \times 1000 \times f$$

Serum:

Definition: At 25°C, the amount of enzyme required to catalyze the generation of 1 μmol of p-nitrophenol per minute per liter of serum in the process of catalyzing the substrate is defined as one enzyme activity unit.

$$\text{LPL activity (U/L)} = (\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) - b \div a \div T \times 1000 \times f$$

[Note]

y: OD value of standard wells - OD value of blank wells (the OD value when the standard concentration is 0)

x: The concentration of the standard

a: The slope of the standard curve

b: The intercept of the standard curve

$\Delta A_{\text{sample}} = A_2 - A_1$ (the A_2 value of the sample well - the A_1 value of the sample well)

$\Delta A_{\text{Blank}} = A_2 - A_1$ (the A_2 value of the blank well - the A_1 value of the blank well)

T: Reaction time (determined according to the specific experimental time)

C_{pr} : Protein concentration of the tissue sample, gprot/L

1000: 1 mmol/L = 1000 $\mu\text{mol/L}$

f: The dilution factor of the sample before being added to the detection system

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver tissue 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)	20	40	60
%CV	5	5	4.5

Inter-assay Precision

Three mouse liver tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	20	40	60
%CV	9	1.8	6.7

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. (U/L)	20	40	60
Observed Conc. (U/L)	20.4	40	56.4
Recovery rate (%)	102	100	94

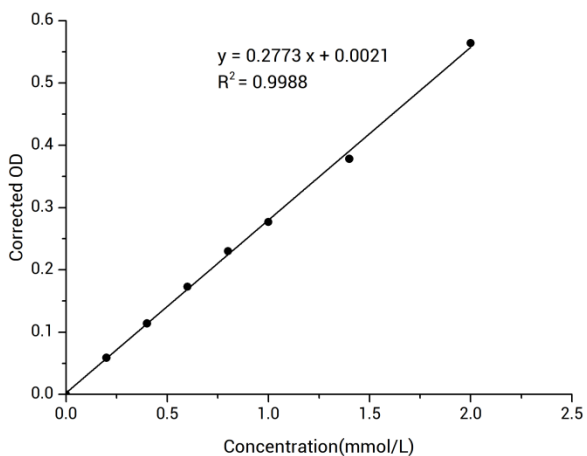
Sensitivity

The analytical sensitivity of the assay is 1.73 U/L. This was determined by adding two standard deviations to the mean OD. 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	0.2	0.4	0.6	0.8	1	1.4	2
OD Value	0.057	0.115	0.170	0.230	0.283	0.327	0.416	0.604
	0.057	0.116	0.171	0.229	0.290	0.340	0.453	0.637
Average OD	0.057	0.116	0.171	0.230	0.287	0.334	0.435	0.621
Absoluted OD	0	0.059	0.114	0.173	0.230	0.277	0.378	0.564



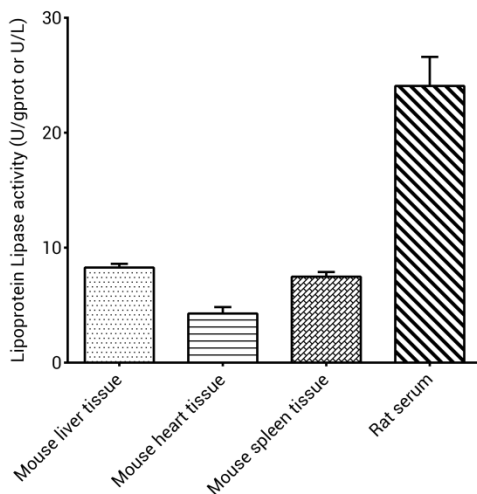
Appendix II Example Analysis

Example analysis:

Take 10 μ L of the 10% mouse liver tissue sample diluted 2-fold and add it into the microplate, and proceed according to the operation table. The results are as follows: the average OD value of blank wells A_1 is 0.143; the average OD value of blank wells A_2 is 0.211, $\Delta A_{\text{Blank}} = 0.211 - 0.143 = 0.068$; the average OD value of test wells A_1 is 0.259; the average OD value of test wells A_2 is 0.441, $\Delta A_{\text{Test}} = 0.441 - 0.259 = 0.182$. The protein concentration of the 10% mouse liver tissue homogenate is 19.34 gprot/L, and the calculation result is:

$$\begin{aligned}\text{LPL activity (U/gprot)} &= [(0.182 - 0.068) - 0.0021] \div 0.2773 \div 5 \div 19.34 \times 1000 \times 2 \\ &= 8.35 \text{ U/gprot}\end{aligned}$$

Detect 10% mouse liver tissue homogenization (the concentration of protein is 19.34 gprot/L, dilute for 2 times), 10% mouse heart tissue homogenization (the concentration of protein is 15.32 gprot/L, dilute for 2 times), 10% mouse spleen tissue (the concentration of protein is 19.63 gprot/L) and rat serum, 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.

