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

Catalog No：E-BC-K786-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader（400-420 nm）

Detection range：0.03-9.41 U/L

Elabscience®Lipase (LPS) 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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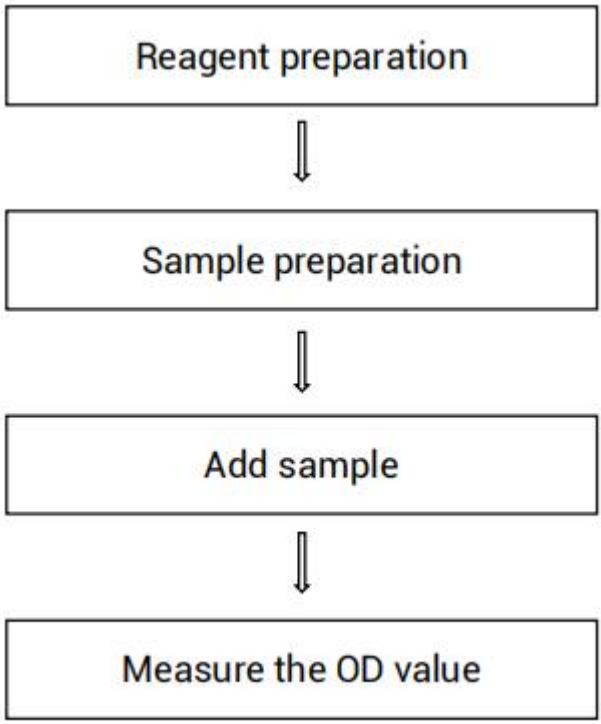
Assay summary



Intended use

This kit can measure lipase (LPS) activity in serum, plasma, animal tissue and cell samples.

Detection principle

Lipase can catalyze the substrate to produce sulfhydryl compounds, which react with DTNB to generate TNB. TNB has the maximum absorption at 412 nm. The activity of LPS in the sample can be calculated by measuring the change of absorbance per unit time.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 2	Inhibitor	0.5 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months
Reagent 3	Substrate	3 mL × 1 vial	5 mL × 1 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent	0.9 mL×1 vial	1.8 mL×1 vial	2-8℃, 12 months, shading light
	Microplate	48 wells	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:

Incubator, Centrifuge, Microplate reader (400-420 nm, optimum wavelength: 412 nm)

Reagents:

Double distilled water, Ethanol absolute (99.5%)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of inhibitor working solution：
Before testing, please prepare sufficient inhibitor working solution according to the test wells. For example, prepare 50 μL of inhibitor working solution (mix well 5 μL of inhibitor and 45 μL of ethanol absolute). Store at 2-8°C for 7 days.

③ The preparation of substrate working solution：
Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 505 μL of substrate working solution (mix well 5 μL of inhibitor working solution and 500 μL of substrate). The substrate working solution should be prepared on spot. The prepared solution should be used up within 1 h.

④ The preparation of chromogenic working solution：
For each well，prepare 150 μL of chromogenic working solution (mix well 15 μL of chromogenic agent and 135 μL of double distilled water). The chromogenic working solution should be prepared on spot. Keep chromogenic working solution protected from light during use.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If the sample is turbidity, centrifuge at 10000×g for 10 min, then take the supernatant for detection. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

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 buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ 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).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL buffer solution with a ultrasonic cell disruptor at 4℃.

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
10% Mouse liver tissue homogenate	6-10
10% Rat brain tissue homogenate	2-6
10% Rat kidney tissue homogenate	3-6
10% Rat liver tissue homogenate	5-10
10% Rat heart tissue homogenate	2-5
10% Rat lung tissue homogenate	3-6
10% Rat spleen tissue homogenate	2-5
10% Mouse lung tissue homogenate	3-6
Human serum	7-10
Human plasma	2-6

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

The key points of the assay

① Prepare the fresh needed amount of substrate working solution before use and the prepared solution should be used up within 1 h.

② It is recommended to add chromogenic working solution under ventilation conditions due to the generated product with certain stimulating odor after incubation reaction

Operating steps

① Control well: Add 10 μL of sample to the corresponding wells.
Sample well: Add 10 μL of sample to the corresponding wells.

② Add 40 μL of buffer solution to control wells, add 40 μL of substrate working solution to sample wells.

③ Mix fully with microplate reader for 5 s and incubate at 37℃ for 20 min.

④ Add 150 μL of chromogenic working solution to each well.

⑤ Mix fully with microplate reader for 5 s and incubate at 37℃ for 30 min with shading light. Measure the OD value of each well at 412 nm with microplate reader.

Calculation

The sample:

1. Serum (plasma) sample:

Definition: The amount of LPS in 1 L liquid sample per 1 minute that hydrolyze the substrate to produce 1 μmol TNB at 37°C is defined as 1 unit

$$\text{LPS activity (U/L)} = \frac{\Delta A}{\varepsilon \times b} \times f \div T \times 10^6$$

2. Tissue and cells sample:

Definition: The amount of LPS in 1 g tissue protein per 1 minute that hydrolyze the substrate to produce 1 μmol TNB at 37°C is defined as 1 unit.

$$\text{LPS activity (U/gprot)} = \frac{\Delta A}{\varepsilon \times b} \div C_{pr} \times f \div T \times 10^6$$

[Note]

ΔA: OD_{Sample} - $OD_{Control}$.

ε: Molar absorption coefficient, 14150 L•mol-1•cm-1.

b: The height of the reaction system, 0.6 cm.

T: The time of incubation reaction, 20 min.

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

f: Dilution factor of sample before test.

10^6: 1 mol/L = 10^6 μmol/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)	0.85	2.40	6.80
%CV	3.3	2.9	2.8

Inter-assay Precision

Three human serum 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)	0.85	2.40	6.80
%CV	5.7	5.9	6.4

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 105%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	1.7	4.6	8.5
Observed Conc. (U/L)	1.8	4.7	9.1
Recovery rate(%)	106	102	107

Sensitivity

The analytical sensitivity of the assay is 0.03 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.

Appendix Π Example Analysis

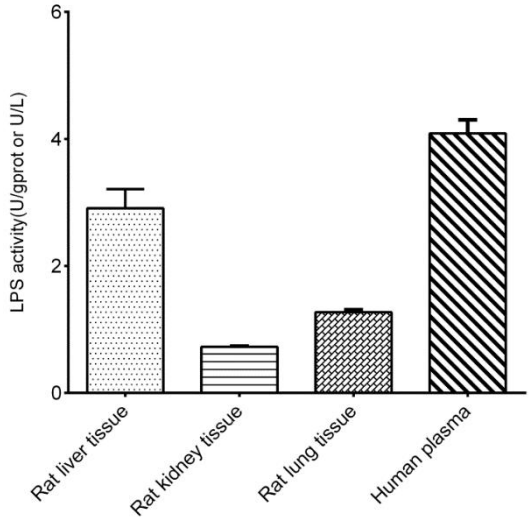
Example analysis：

For 10% rat liver tissue homogenate, dilute for 5 times, and carry the assay according to the operation steps. The results are as follows:

The average OD value of the control is 0.429, the average OD value of the sample is 1.513, ΔA=1.513 - 0.429 = 1.084, the concentration of protein in sample is 11.00 gprot/L, and the calculation result is:

LPS activity (U/gprot) = 1.084 × (14150 × 0.6) ÷ 11.00 × 5÷ 20 × 106 = 2.90 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 11.00 gprot/L, dilute for 5 times), 10% rat kidney tissue homogenate (the concentration of protein is 9.57 gprot/L, dilute for 5 times), 10% rat lung tissue homogenate (the concentration of protein is 6.03 gprot/L, dilute for 5 times), and human plasma according to the protocol, the result is as follows：



Appendix III Publications

1. Ouyang B, Zhong Q, Ouyang P, et al. Graphene quantum dots enhance the biological nitrogen fixation by up-regulation of cellular metabolism and electron transport[J]. Chemical Engineering Journal, 2024, 487: 150694.
2. Wang X, Wang Y, Huo H, et al. Transient receptor vanilloid subtype 4-mediated Ca2+ influx promotes glomerular endothelial inflammation in sepsis-associated acute kidney injury[J]. Laboratory Investigation, 2023, 103(6): 100126.
3. Zhao X, Cai P, Xiong S, et al. Lacticaseibacillus rhamnosus NCUH061012 alleviates hyperuricemia via modulating gut microbiota and intestinal metabolites in mice[J]. Food Bioscience, 2024, 58: 103699.
4. Genc E, Kaya D, Genc M A, et al. Effect of biofloc technology in Farfantepenaeus aztecus culture: The optimization of dietary protein level on growth performance, digestive enzyme activity, non - specific immune response, and intestinal microbiota[J]. Journal of the World Aquaculture Society, 2024, 55(2): e13041.
5. Genc E, Kaya D, Genc M A, et al. Effect of dietary mannan oligosaccharide inclusion on production parameters of Farfantepenaeus aztecus cultured in a biofloc system[J]. Journal of the World Aquaculture Society, 2024, 55(5): e13086.
6. Fu S, Qian K, Tu X, et al. Comparative analysis of intestinal structure, enzyme activity, intestinal microbiota and gene expression in different segments of pufferfish (Takifugu Obscurus)[J]. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 2024, 52: 101341.

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.