TECHNICAL MANUAL

Lipase (LPS) Activity Assay Kit

- **SKU CODE:** MAES0218
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Add sample to wells
- Add buffer solution or substrate working solution
- Incubate at 37°C for 20 min
- Add chromogenic working solution
- Incubate at 37°C for 30 min with light protection
- Read absorbance at 412 nm
- ![img-0.jpeg](img-0.jpeg)

2. Intended use

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

3. Detection principle

Lipase can catalyze the lipid 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.

4. 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, protect from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Ethanol absolute (99.5%)

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 to ensure sufficient amount of reagents.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of inhibitor working solution:** Before testing, 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. **Preparation of substrate working solution:** Before testing, 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 fresh. The prepared solution should be used up within 1 hour.
4. **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 fresh. Keep chromogenic working solution protected from light during use.

7. Sample preparation

Sample preparation

Serum and plasma: Detect directly. If the sample is turbid, centrifuge at 10,000×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 10,000×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 (MAES0177).

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 an ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10,000×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 (MAES0177).

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 pre-test to confirm the dilution factor.

8. 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 hour.

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

9. 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 light protection. Measure the OD value of each well at 412 nm with microplate reader.

10. Calculation

The sample:

1. Serum (plasma) sample:

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

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

2. Tissue and cells sample:

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

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

[Note]

ΔA: OD_{Sample} - $OD_{Control}$.

ε: Molar absorption coefficient, 14,150 $L \bullet mol^{-1} \bullet cm^{-1}$.

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, g_{prot}/L.

f: Dilution factor of sample before test.

10^6: 1 mol/L = 10^6 µmol/L.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

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%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	1.7	4.6	8.5

	Sample 1	Sample 2	Sample 3
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.

12. Appendix II Example Analysis

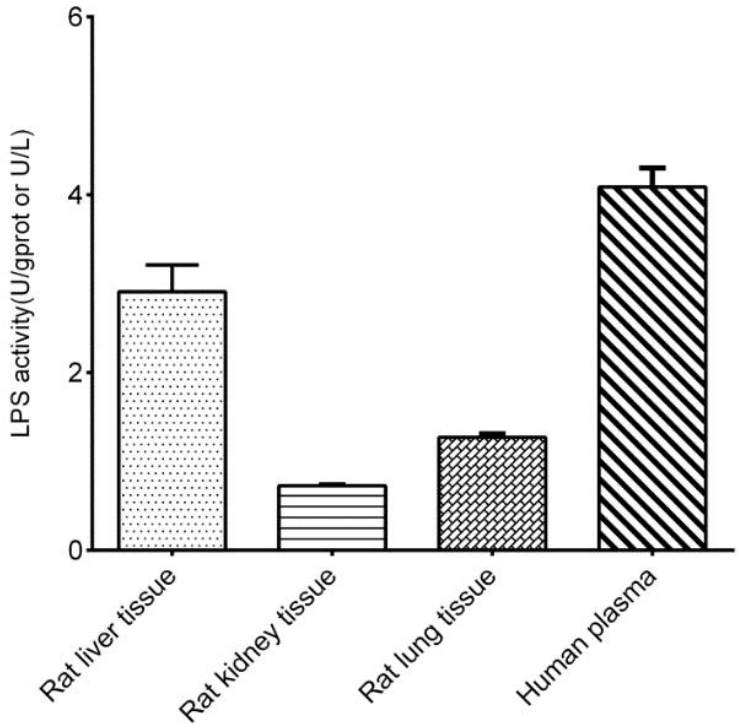
Example analysis:

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 g_{prot}/L, and the calculation result is:

LPS activity (U/g_{prot}) = 1.084 × (14,150 × 0.6) ÷ 11.00 × 5÷ 20 × 10^6 = 2.90 U/g_{prot}

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



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.