TECHNICAL MANUAL

Lipid Peroxide (LPO) Colorimetric Assay Kit

- **SKU CODE:** MAES0132
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Add chromogenic working solution
- Add acid reagent
- Incubate at 45°C for 60 min
- Centrifuge and measure OD values
- ![img-0.jpeg](img-0.jpeg)

2. Intended use

This kit can be used to measure LPO content in serum, plasma, urine, and tissue samples.

3. Detection principle

With 45°C incubation for 60 min, one molecule of LPO reacts with two molecules of chromogenic reagent to produce a stable chromophore which has a maximum absorption peak at 586 nm. The content of LPO in samples can be calculated using the standard curve or calculation formula.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Substrate Stock Solution	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Diluent	20 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Acid Reagent	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	100 μmol/L Standard	6 mL ×1 vial	6 mL ×1 vial	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (580-590 nm, optimum wavelength: 586 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer.

Reagents:

Anhydrous ethanol, PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all the reagents to room temperature before use.
2. **Preparation of chromogenic working solution::** For each well, prepare 650 µL of chromogenic working solution (mix well 487.5 µL of substrate stock solution and 162.5 µL of diluent). The chromogenic working solution should be prepared fresh on the day of use.
3. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 µmol/L standard solution with absolute ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 30, 40, 50, 80 µmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Urine: Collect fresh urine and centrifuge at 10,000×g for 15 min at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

Tissue samples:

1. Harvest the amount of tissue needed for each assay (initial recommendation 40 mg).

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

3. Homogenize 40 mg tissue in 360 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000×g for 10 min 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):

- Human serum: 1

- Human plasma: 1

- Urine: 1

- 10% Rat kidney tissue homogenization: 1

- 10% Rat liver tissue homogenization: 1

Note: The diluent is PBS (0.01 M, pH 7.4). For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

1. The EP tube needs to be sealed to avoid leakage.

2. The supernatant added to 96-well microplate must be clarified, otherwise centrifuge again.

3. Please carry out the experiment in the fume hood and wear disposable gloves to prevent the reagents from splashing into eyes and skin.

9. Operating steps

1. Standard well: add 200 μL of standard solution with different concentrations into 1.5 mL EP tubes. Sample well: add 200 μL of sample into 1.5 mL EP tubes.

2. Add 650 μL of chromogenic working solution, cover the caps and mix fully.

3. Add 150 μL of acid reagent, cover the caps and mix fully.

4. Incubate at 45°C for 60 min. Cool to room temperature with running water.

5. Centrifuge at 1,100×g for 10 min. Take 200 μL of supernatant to the microplate, measure the OD values of each well at 586 nm with microplate reader. (Avoid bubbles when adding liquid to microplate, otherwise OD value will be affected.)

10. 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:

1. Serum (plasma) and other liquid sample:

LPO (μmol/L) = (ΔA_586 - b) ÷ a × f

2. Tissue sample (Calculated by tissue protein):

LPO (μmol/g_{prot}) = (ΔA_586 - b) ÷ a × f ÷ C_{pr}

3. Tissue sample (Calculated by tissue wet weight):

LPO (μmol/kg wet weight) = (ΔA_586 - b) ÷ a × f ÷ m × V

[Note]

ΔA_586: Absolute OD (OD_{Sample} – OD_{Blank}).

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (g_{prot}/L).

m: The wet weight of tissue, g.

V: The volume of homogenate of tissue sample, mL.

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 (μmol/L)	5.60	34.50	66.00
%CV	3.5	2.9	2.9

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 (µmol/L)	5.60	34.50	66.00
%CV	3.2	3.5	3.8

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration measured 6 times in parallel to get the average recovery rate of 99%.

Recovery

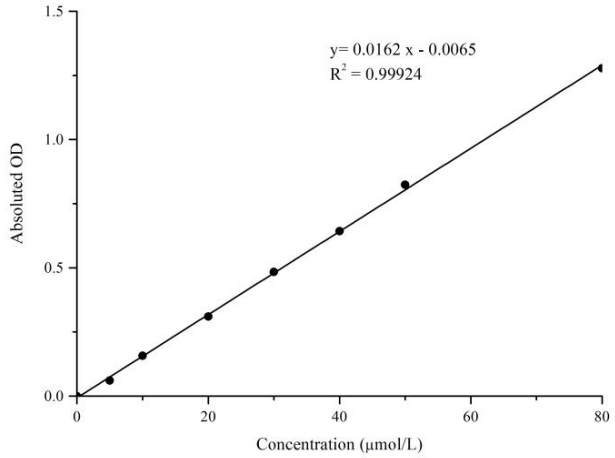
Sample	Expected Conc. (µmol/L)	Observed Conc. (µmol/L)	Recovery rate (%)
Standard 1	7.5	7.6	101
Standard 2	26.4	26.1	99
Standard 3	48.5	47.0	97

Sensitivity

The analytical sensitivity of the assay is 0.70 µmol/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.

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), the standard curve and data are provided below for reference only.



Standard curve

Concentration (μmol/L)	Average OD	Absolute OD
0	0.079	0
5	0.140	0.061
10	0.236	0.157
20	0.389	0.31
30	0.563	0.484
40	0.722	0.643
50	0.903	0.824
80	1.357	1.278

12. Appendix II Example Analysis

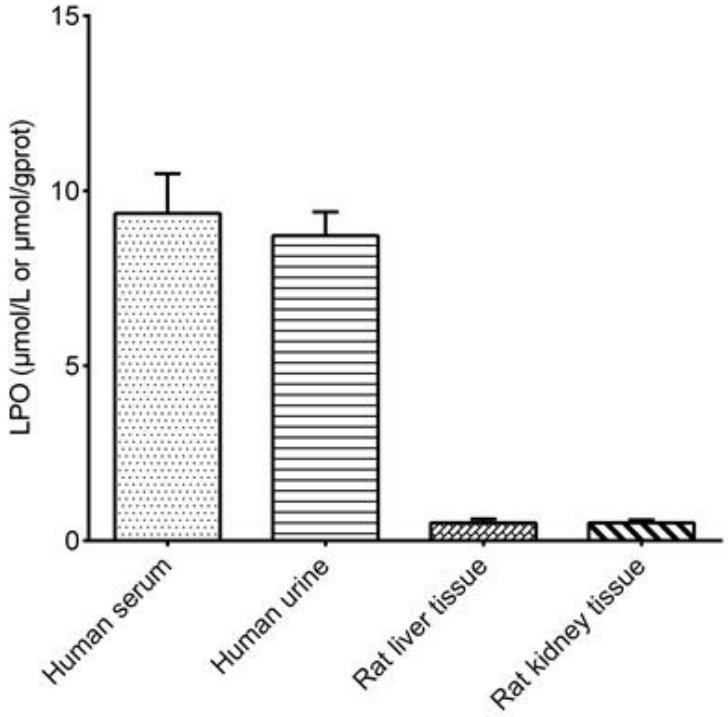
Example analysis:

Take 200 μL of human serum and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0162x - 0.0065, the average OD value of the sample is 0.219, the average OD value of the blank is 0.073, and the calculation result is:

LPO (μmol/L) = (0.219 - 0.073 + 0.0065) ÷ 0.0162 × 1 = 9.41 (μmol/L)

Detect human serum, human urine, 10% rat liver tissue homogenate (the concentration of protein is 13.10 g_{prot}/L), 10% rat kidney tissue homogenate (the concentration of protein is 9.26 g_{prot}/L) 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.