IMMUNOLOGICAL SCIENCES Product Data Sheet

CO-K176-M Lipid Peroxide (LPO) Colorimetric Assay Kit

Method: Colorimetric method

Specification：96T (80 samples)

Measuring instrument：Microplate reader（580-590 nm）

Detection range：0.70-80 μmol/L

Intended use

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

Detection principle

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

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 ℃, 12 months, shading light
Reagent 2	Diluent	20 mL ×1 vial	20 mL ×1 vial	2-8 ℃, 12 months, shading light
Reagent 3	Acid Reagent	10 mL ×1 vial	20 mL ×1 vial	2-8 ℃, 12 months, shading light
Reagent 4	100 μmol/L Standard	6 mL ×1 vial	6 mL ×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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The 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 on spot.

③ The 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**20**	**30**	**40**	**50**	**80**
100 μmol/L standard (μL)	0	50	100	200	300	400	500	800
Absolute ethanol (μL)	1000	950	900	800	700	600	500	200

Sample preparation

① Sample preparation:

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

Urine: Collect fresh urine and centrifuge at 10000×g for 15 min at 4 ℃. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80 ℃ 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 ℃.

④ Centrifuge at 10000×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

② Dilution of sample

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

Sample type	Dilution factor
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 do pretest to confirm the dilution factor.

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, prevent the reagents splashing dashed into eyes and skin.

Operating steps

① Standard well: add 200 μL of standard solution with different concentration into the 1.5 mL EP tube.
Sample well: add 200 μL of sample into the 1.5 mL EP tube.

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 ℃ for 60 min. Cool to room temperature with running water.

⑤ Centrifuge at 1100×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 the bubbles generated when adding the liquid to microplate, otherwise OD value will be affected.)

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 absoluted OD value.

3. Plot the standard curve by using absoluted 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:

$$\text{LPO (μmol/L)} = (\Delta A_{586} - b) \div a \times f$$

2. Tissue sample (Calculated by tissue protein):

$$\text{LPO (μmol/gprot)} = (\Delta A_{586} - b) \div a \times f \div C_{pr}$$

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

$$\text{LPO (μmol/kg wet weight)} = (\Delta A_{586} - b) \div a \times f \div m \times V$$

[Note]

ΔA_{586}: Absolute OD ($OD_{Sample} - OD_{Blank}$).

f: Dilution factor of sample before test.

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

m: The wet weight of tissue, g.

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

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 (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.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	5.60	34.50	66.00
%CV	3.2	3.5	3.8

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. (μmol/L)	7.5	26.4	48.5
Observed Conc. (μmol/L)	7.6	26.1	47.0
Recovery rate (%)	101	99	97

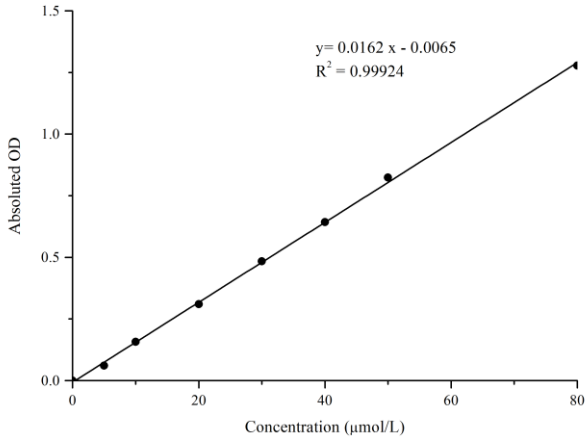
Sensitivity

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.

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



Appendix II Example Analysis

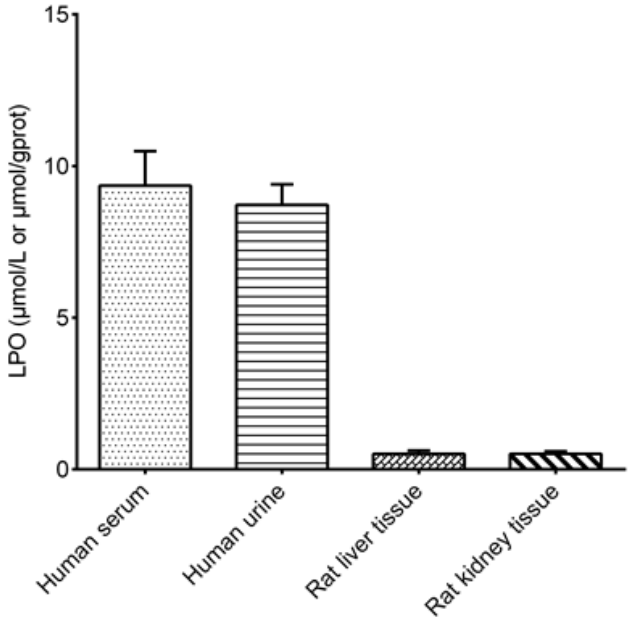
Example analysis:

Take 200 μL of human serum, carry 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:

$$\text{LPO (μmol/L)} = (0.219 - 0.073 + 0.0065) \div 0.0162 \times 1 = 9.41 \text{ (μmol/L)}$$

Detect human serum, human urine, 10% rat liver tissue homogenate (the concentration of protein is 13.10 gprot/L), 10% rat kidney tissue homogenate (the concentration of protein is 9.26 gprot/L) 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. Immunological Sciences 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.