Technical Manual

Lipid Peroxide (LPO) Colorimetric Assay Kit

- Catalogue Code: MAES0132
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.70-80 μmol/L

Sensitivity:

0.70 μmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background:

Lipid peroxidation, as an indicator of oxidative stress in cell and tissue, has been identified as a kind of cellular damage. Lipid peroxide is unstable and can decompose into complex mixture including carbonyl compounds. Polyunsaturated fatty acid peroxide is decomposed into Malondialdehyde (MDA) and 4- hydroxyl olefins (HAE). Detection of LPO, MDA and HAE has been an indicator of lipid peroxidation.

3. Intended Use:

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

4. Detection Principle:

With 45°C 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 586nm. The content of LPO in samples can be calculated by standard curve or calculation formula.

5. Kit Components & Storage:

Item	Specification	Storage
Substrate Stock Solution	60 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight.
Diluent	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight.
Acid Reagent	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight.
Standard (100 μmol/L)	6 mL ×1 vial	2-8°C, 6 months, avoid direct sunlight.
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (580-590 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

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

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

3. Carry out the experiment in the fume hood and wear disposable gloves, prevent the reagents splashing dashed into eyes and skin.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **chromogenic agent working solution:** Mix the substrate stock solution and diluent at the ratio of 3:1 thoroughly. Prepare the fresh solution before use.

8. Sample Preparation:

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Urine:
Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the urine can be stored at -80°C for a month.

4. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb water and weigh. Homogenize at the ratio of the volume of PBS (0.01 M，pH 7.4) or 20 mM Tris-HCl（pH 7.4） (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.70-80 μmol/L).

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).

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 586 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps:

The preparation of standard curve

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.

The measurement of samples

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 agent 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. (Prevent the bubbles generated when adding the liquid to microplate, otherwise OD value will be affected.)

Operation Table

	Standard well	Sample well
Sandard solution with different concentration (μL)	200	
Sample (μL)		200
Cover the caps and mix fully.		
Chromogenic agent working solution (μL)	650	650
Acid reagent (μL)	150	150
Cover the caps and mix fully, incubate at 45°C for 60 min. Cool to room temperature with running water. Centrifuge at 1100 g for 10 min. Take 200 μL of supernatant to the microplate, measure the OD value of each well at 586 nm with microplate reader.		

11. Calculations:

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) sample:

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

2. Tissue sample (Calculated by tissue protein):

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

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

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

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)
x: The concentration of standard
a: The slope of standard curve
b: The intercept of standard curve
ΔA_{586}: $OD_{Sample} - OD_{Contorl}$
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

12. Performance Characteristics:

Detection Range	0.70-80 μmol/L
Sensitivity	0.70 μmol/L
Average recovery rate (%)	99
Average inter-assay CV (%)	3.5
Average intra-assay CV (%)	3.1

Analysis

Take 200 μL of human serum, then take prepared supernatant, carry the assay according to the operation table.

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)

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Email: info@assaygenie.com **Web:** www.assaygenie.com

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Email: info@assaygenie.com **Web:** www.assaygenie.com

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