PRODUCT INFORMATION & MANUAL

Lipid Peroxide/LPO Assay Kit (Colorimetric)

NBP3-25862

For research use only.
Not for diagnostic or therapeutic procedures.

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Lipid Peroxide (LPO) Colorimetric Assay Kit

Catalog No: NBP3-25862

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.70 μmol/L

Detection range: 0.70-80 μmol/L

Average intra-assay CV (%): 3.1

Average inter-assay CV (%): 3.5

Average recovery rate (%): 99

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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 586nm. The content of LPO in samples can be calculated by standard curve or calculation formula.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Substrate Stock Solution	60 mL × 1 vial	2-8℃, 12 months, shading light.
Reagent 2	Diluent	20 mL × 1 vial	2-8℃ ,12 months, shading light.
Reagent 3	Acid Reagent	20 mL × 1 vial	2-8℃ ,12 months, shading light.
Reagent 4	100 μmol/L Standard	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.			

▲Materials prepared by users

Instruments

Microplate reader (580-590 nm), Water bath, Centrifuge, Micropipettor, Vortex mixer

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ 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.

Pre-assay preparation

Reagent preparation

Preparation of chromogenic agent working solution

Mix the reagent 1 and reagent 2 at the ratio of 3:1 thoroughly. Prepare the fresh solution before use.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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) .

Assay protocol

▲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, blank wells; B-H, standard wells; S1-S80, sample wells.

▲Detailed operating 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. Reference is as follows:

Number	Standard concentrations (μmol/L)	100 μmol/L Standard (μL)	Absolute ethanol (μL)
A	0	0	1000
B	5	50	950
C	10	100	900
D	20	200	800
E	30	300	700
F	40	400	600
G	50	500	500
H	80	800	200

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 reagent 3, 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 value 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.)

▲Summary 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
Reagent 3 (μL)	150	150
Cover the caps and mix fully, incubate at 45 ℃ for 60 min. Cool to room temperature with running water. Centrifuge for 10 min. Take 200 μL of supernatant to the microplate, measure the OD value at 586 nm.		

▲Calculation

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. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

Note:

y: The absolute OD value of standard

x: The concentration of Standard

a: The slope of standard curve

b: The intercept of standard curve

1. Serum (plasma) and other liquid sample:

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

2. Tissue sample (Calculated by tissue protein) :

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

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

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

Note:

ΔA_{586}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

Cpr: 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 Data

▲Example analysis

Take 200 μL of human serum, 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)

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

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

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the 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 sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 20 mM Tris-HCl(pH 7.4).

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2)Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3)Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Devasagayam T P, Boloor K K, Ramasarma T. Methods for estimating lipid peroxidation: an analysis of merits and demerits[J]. Indian J Biochem Biophys, 2003, 40(5): 300-308.
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3. Reed T T. Lipid peroxidation and neurodegenerative disease[J]. Free Radical Biology & Medicine, 2011, 51(7): 1302-1319.
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5. Niki E. Biomarkers of lipid peroxidation in clinical material[J]. Biochim Biophys Acta, 2014, 1840(2): 809-817.