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Lipid Peroxidation (LPO)Assay Kit

CAT/NO.: BC015 Pack: 50T/100T

I. Reagent Composion and Preparation:

1、Reagent Composion（50T/48S）:

Reagent 1: Substance Stock solution, 30ml×1 bottle; Dilution, 10ml×1 bottle; stored at 2~8℃ for 1 year;**Prepare of Reagent 1 Working Solution:** Stock solution：Dilution=3：1，prepare when you need;

Reagent 2: LPO Developer；10ml×1 bottle;stored at 2~8℃ for 1 year;

Reagent 3： 10μmol/L Standard Solution；5ml×1 bottle.

2、Reagent Composion（100T/96S）:

Reagent 1: Substance Stock solution, 60ml×1 bottle; Dilution, 20ml×1 bottle; stored at 2~8℃ for 1 year;**Prepare of Reagent 1 Working Solution:** Stock solution：Dilution=3：1，prepare when you need;

Reagent 2: LPO Developer；18ml×1 bottle;stored at 2~8℃ for 1 year;

Reagent 3： 10μmol/L Standard Solution；5ml×1 bottle.

II. Operation procedures:

1、Sample pretreatments:

Blood serum (or plasma) can be assay directly

Tissue： Weigh tissue to assay accurately, add physiological saline to make 10% tissue homogenate by homogenate machine or homogenate tube according to mass-volume ratio (tissue：physiological saline = 1g：9ml). Centrifuge at 2500 rpm for 10 minutes, take supernatant to assay.

2、Operation table:

	Blank	Standard	Assay
Absolute Alcohol (μl)	200		
10μmol/L Standard Solution (μl)		200	
Sample (μl)			200
Reagent 1 Working Solution (ul)	650	650	650
Cover and mix well			
Reagent 2 (μl)	150	150	150

Mix well, incubate at 45℃ for 60 minutes, Centrifuge at 4000 rpm for 10 minutes, take supernatant 200 μl to 96 orifice plate to detect the OD at 586nm.

Attention：Please take the gloves and not splash the reagents to eyes and skin.

III. Calculating formula：

Blood（Plasma）：

$$\underset{(\mu mol/L)}{\text{Blood LPO concent}} = \frac{OD_{Assay} - OD_{Blank}}{OD_{S\,tan\,dard} - OD_{Blank}} \times \underset{(10\,\mu mol/L)}{\text{Standard concentration}}$$

Tissue：

$$\underset{(\mu mol/gprot)}{\text{Tissue LPO content}} = \frac{OD_{Assay} - OD_{Blank}}{OD_{S\,tan\,dard} - OD_{Blank}} \times \underset{(10\,\mu mol/L)}{\text{Standard concention}} \div \underset{gprot/L}{\text{Protein concentration}}$$

IV. Assay principle：

Under condition of 45℃ and 60 minutes, a molecule lipid peroxide reacts with 2 molecule chromogenic agent to produce a stable chromophore. This chromophore has maximum absorption peak at 586nm. Sample lipid peroxide content is available by comparing with standard curve or formula calculations.

V. Assay significance：

In organisms, many lipids contain unsaturated fatty acids, especially, biomembranous phospholipid contains extremely large amount of unsaturated fatty acids.Unsatured fatty acids have instable chemical properties, they can be easily damaged by peroxidation and produce fatty acids lipid peroxides. According to researches for the past few years, lipid peroxides can destroy normal physiologic functions of human body cells, they have close correlations with pathologic processes of some diseases such as tumor, chemical poisoning, infection, inflammation, autoimmune diseases, atherosclerosis (AS), cardiovascular & cerebrovascular diseases, and physiological processes such as aging, etc.

Lipid peroxidation is confirmed as a cell injury mechanism, it is a index for oxidative stress in cells and tissues. Lipid peroxides are instable, they decompose to produce a series of complex mixture include reacting carbonyl mixture. Polyunsaturated lipid peroxides decompose to produce malondialdehyde (MDA) and 4-hydroxyl alkenes (HAE). Measurements of LPO, MDA and HAE are already used as lipid peroxidation indexes.

VI. Announcements：

1、Do not smoke, eat or drink when you treat samples and reagents;

2、Please wear disposable gloves when you treat samples and reagents;

3、Never move reagents or samples by you mouth;

4、If Reagent 1 or Reagent 2 splashes to your skin, mucous membrane or eyes uncarefully, please use water to wash the sites wet by reagents sufficiently.

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Appendix I: LPO Standard Curve Preparation

1、Pretreatment:

Dilute 10μmol/L LPO standard solution with dehydrated alcohol to different concentrations: 5μmol/ L、2.5μmol/ L 、1μmol/ L、0.5μmol/ L、0μmol/ L.

2. Operation table:

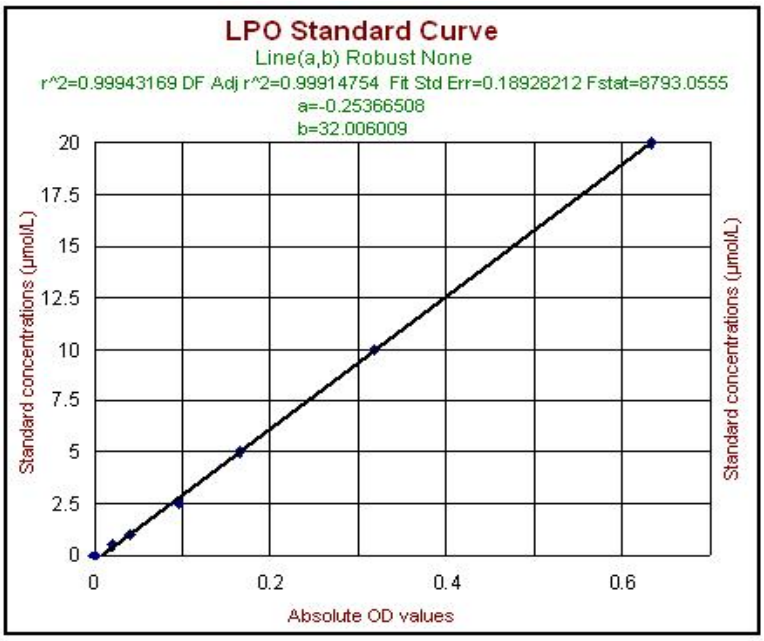
	Blank	Standard
Absolute Alcohol (μl)	200	
10μmol/L Standard Solution (μl)		200
Reagent 1 Working Solution (ul)	650	650
Cover and mix well		
Reagent 2 (μl)	150	150

Mix well, incubate at 45℃ for 60 minutes, Centrifuge at 4000 rpm for 10 minutes, take supernatant 200 μl to 96 orifice plate to detect the OD at 586nm.

3、Results：

Standard concentration（μmol/L）	Assay OD	Absolute OD
0	0.0553	0
0.5	0.0625	0.0072
1	0.0722	0.0169
2	0.094	0.0387
5	0.1539	0.0986
10	0.2552	0.1999

4、Draw graph：



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Appendix II: Tissue LPO Assay

1、Pretreatment:

Weigh 0.2g tissue sample accurately, add 1.8ml physiological saline (Mass(g) : Volume(ml) = 1 : 9), make homogenate in icewater bath, centrifuge at 2500rpm for 10 minutes, take supernatant to operate.

2、Operation table:

	Blank	Standard	Assay
Absolute Alcohol (μl)	200		
10μmol/L Standard Solution (μl)		200	
10% tissue supernatant (μl)			200
Reagent 1 working solution (μl)	650	650	650
Cover and mix well			
Reagent 2 (μl)	150	150	150

Mix well, incubate at 45℃ for 60 minutes, Centrifuge at 4000 rpm for 10 minutes, take supernatant 200 μl to 96 orifice plate to detect the OD at 586nm.

3、Formula:

$$\underset{(\mu\text{mol/gprot})}{\text{Tissue LPO content}} = \frac{OD_{Assay} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{(10\,\mu\text{mol/L})}{\text{Standard concention}} \div \underset{\text{gprot/L}}{\text{Protein concentration}}$$

4、Example：

Take 200μl 10% mouse liver tissue homogenate, operate according to LPO operation table above. In results, ODBlank is 0.0553, ODStandard is 0.2552, ODSample is 0.3155, protein concentration in 10% mouse liver tissue homogenate is 18.8137gprot/L, calculate as follows:

$$\underset{(\mu\text{mol/gprot})}{\text{Tissue LPO content}} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{(10\mu\text{mol/L})}{\text{Standard concentration}} \div \underset{(\text{gprot/L})}{\text{Protein concentration}}$$

$$= \frac{0.3155-0.0553}{0.2552-0.0553} \times 10 \div 18.8137$$

$$= 0.6919\ \mu\text{mol/gprot}$$

Appendix III: Blood Serum LPO Assay

1、Pretreatment:

Take 200μl blood serum to operate directly.

2、Operation table:

	Blank	Standard	Assay
Absolute Alcohol (μl)	200		
10μmol/L Standard Solution (μl)		200	
Human blood (μl)			200
Reagent 1 working solution (μl)	650	650	650
Cover and mix well			
Reagent 2 (μl)	150	150	150

Mix well, incubate at 45℃ for 60 minutes, Centrifuge at 4000 rpm for 10 minutes, take supernatant 200 μl to 96 orifice plate to detect the OD at 586nm.

3、Formula:

$$\underset{(\mu\,\mathrm{mol/L})}{\text{Blood LPO concention}} = \frac{OD_{Assay} - OD_{Blank}}{OD_{S\,\mathrm{tan}\,dard} - OD_{Blank}} \times \underset{(10\,\mu\,\mathrm{mol/L})}{\text{Standard concention}}$$

4、Example：

Take 200μl human blood serum, operate according to LPO operation table above. In results, ODBlank is 0.0553, ODStandard is 0.2552, ODSample is 0.1332, calculate as follows:

$$\text{Human blood serum LPO content } (\mu\text{mol/L}) = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{(10\mu\text{mol/L})}{\text{Standard concentration}}$$

$$= \frac{0.1332-0.0553}{0.2552-0.0553} \times 10$$

$$= 3.8969\,\mu\text{mol/L}$$