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Lipid Peroxide (LPO) Assay Kit

Catalog No.：BC00021

Size：100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email（Sale） order@enkilife.com

✉Email（Techsupport） techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

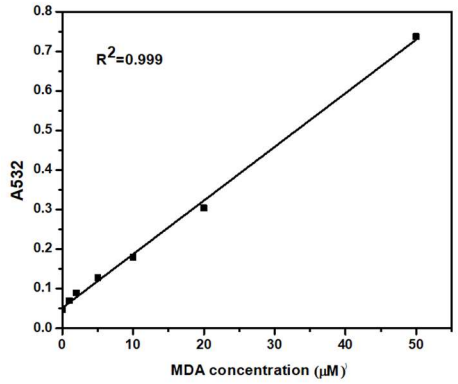
Product Name	Lipid Peroxide (LPO) Assay Kit
Detection Methods	Colorimetric
Sample type	Tissues, cells, serum, plasma, urine
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (530-540 nm, optimal detection wavelength 532 nm. Dual wavelength determination reference wavelength 450 nm)

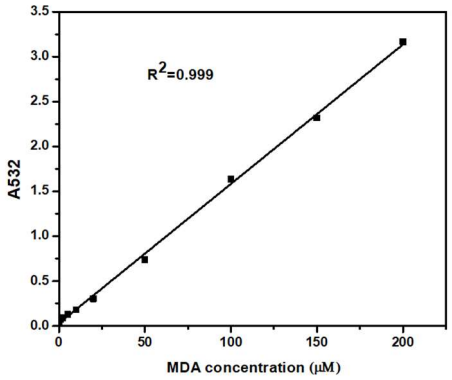
Product Introduction

Lipid peroxides are peroxides produced by unsaturated fatty acid chains after being acted upon by free radicals or reactive oxygen species. Under pathological conditions, the enhanced lipid peroxidation reaction can lead to an increase in the originally low LPO content. An increase in LPO content can damage the structure and function of cells, and LPO content is closely related to the body's immune system and aging.

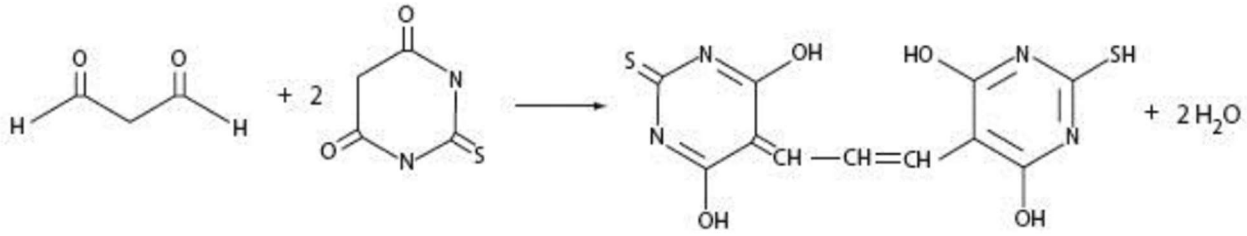
Features

★This kit can measure LPO in the range of 1-200μM. The figure below shows the A532 readings of different concentrations of the standard.





Detection principle



MDA TBA MDA-TBA Adduct

LPO is heated under acidic conditions to produce malondialdehyde, which condenses with thiobarbituric acid (TBA) to produce a reddish-brown substance, trimethoate (3,5,5-trimethyloxazole-2,4-dione), with a maximum absorption wavelength of 532nm. The LPO content in the sample can be estimated by colorimetry.

Product composition

Serial Number	Product Name	Packing Specifications (100T)	Storage
Reagent 1	TBA	25mg	-20℃, keep away from light, after opening the bottle can be stored at room temperature or 2-8 degrees for three months.
Reagent 2	TBA preparation solution	6.76ml	-20℃, after opening the bottle can be stored at room temperature or 2-8 degrees for three months.
Reagent 3	TBA diluent	15ml	-20℃, after opening the bottle can be stored at room temperature or 2-8 degrees for three months.
Reagent 4	Antioxidants	0.3ml	-20℃, keep away from light.
Reagent 5	Standard (1 mM)	0.2ml	-20℃
Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months.

Preparation before the experiment

Sample processing

1. Plasma, serum or urine samples can be directly used for LPO determination after preparation.
2. Tissues or cells can be homogenized or lysed using PBS or lysis buffer. For tissues, the ratio of tissue weight to homogenate or lysate is 10%; for cells, 0.1 ml lysis buffer or homogenate is used for every 1 million cells. After homogenization or lysis, centrifuge at 10000g-12000g for 10 minutes to obtain the supernatant for subsequent determination. If a clear supernatant solution cannot be obtained by centrifugation, or if turbidity occurs after adding LPO detection working solution, filter it with a 0.2 micron pore size filter to obtain a clear sample solution. Sample preparation steps such as homogenization or lysis should be performed in an ice bath or at 4ºC.
3. For tissue or cell samples, after sample preparation, the protein concentration can be measured using the protein concentration determination kit (BCA method) (BC00006) produced by EnkiLife, so as to facilitate the subsequent calculation of the LPO content in the tissue or cell per unit protein weight.

· Preparation of the kit

1. Preparation of TBA stock solution: Weigh an appropriate amount of TBA and use TBA preparation solution to prepare a TBA stock solution with a concentration of 0.37%. For example, 25 mg of TBA is prepared with 6.76 ml of TBA preparation solution, and the final concentration is 0.37%. The TBA preparation solution must be completely dissolved before use, and it can be heated to 70 ºC to promote dissolution. TBA storage solution is difficult to dissolve and needs to be heated to 70 ºC and vigorously shaken to promote dissolution. The prepared TBA storage solution should be stored at room temperature away from light and is valid for at least 3 months.
2. Preparation of LPO detection working solution: According to the number of samples to be tested (including controls), refer to the table below to freshly prepare an appropriate amount of LPO detection working solution just before the test.

Number of Detections	1 time	10 times	20 times	50 times
TBA diluent	150μl	1500μl	3000μl	7500μl
TBA storage solution	50μl	500μl	1000μl	2500μl
Antioxidants	3μl	30μl	60μl	150μl

Note: LPO working solution is difficult to dissolve. It can be heated at 70°C and shaken vigorously to promote dissolution. It can also be sonicated to promote dissolution. The LPO

working solution used for the test standards and test samples should be prepared in the same batch or using the same preparation method. The prepared LPO working solution must be used on the same day.

3. Dilution of standard: Take appropriate amount of standard and dilute it with distilled water to 1, 2, 5, 10, 20, 50μM for subsequent preparation of standard curve. If the concentration of LPO in the sample is very high, you can increase the concentration of standard to 100, 150 and 200μM.

Operation process

1. Add 0.1ml of homogenate, lysate or PBS as a blank control in a centrifuge tube or other appropriate container, add 0.1ml of the above standards of different concentrations to make a standard curve, add 0.1ml of sample for determination, and then add 0.2ml of LPO detection working solution. Refer to the table below to set up the detection reaction system.
2. After mixing, heat at 100ºC or in a boiling water bath for 15 minutes. Be careful not to splash the liquid when heating. If you use a heat block for heating, be sure to press the centrifuge tube lid tightly with a heavy object; if you use a boiling water bath, you need to use a centrifuge tube with a locked lid or a screw cap centrifuge tube, or seal the centrifuge tube mouth with Parafilm and pierce a small hole with a needle. The most convenient and accurate heating method is to use a PCR instrument with a hot lid that can heat 0.5ml PCR tubes.
3. Cool to room temperature in a water bath and centrifuge at 1000g for 10 minutes at room temperature. Take 200 microliters of supernatant and add it to a 96-well plate. Then use an ELISA reader to measure the absorbance at 532nm. You can also measure the absorbance between 530-540nm. You can set 450nm as the reference wavelength for dual wavelength measurement.

The operation table is as follows:

	Blank control	**Standards**	**Sample**
Homogenate, lysate or PBS	0.1ml	—	—
Standards at different concentrations	—	0.1ml	—
Samples to be tested	—	—	0.1ml
LPO detection working fluid	0.2ml	0.2ml	0.2ml
After mixing, heat at 100ºC or in a boiling water bath for 15 minutes. Cool to room temperature in a water bath and centrifuge at 1000g for 10 minutes at room temperature. Take 200 μl of supernatant and add it to a 96-well plate. Measure the absorbance of each			

well at 532 nm with an ELISA reader.

Result Calculation

For samples such as plasma, serum or urine, the molar concentration of LPO can be directly calculated based on the standard curve. For cells or tissue samples, after calculating the LPO content in the sample solution, the LPO content in the initial sample can be expressed by the protein content per unit weight or tissue weight, such as μmol/mg protein or μmol/mg tissue.

Notes

1. No LPO was detected. The LPO concentration in the sample may be too low and below the detection limit. When testing LPO in tissues or cells, please use more tissues or cells. And be careful not to dilute the sample as much as possible.
2. Because the LPO detection working solution is unstable, it is recommended to make a standard curve each time, or to use the same conditions when processing standards and samples.
3. This product is limited to scientific research by professionals and must not be used for clinical diagnosis or treatment, used as food or medicine, or stored in ordinary residences.