Lipid Peroxidation MDA Assay Kit (TBA Method)

Catalog No.: K084

Size: 100T/500T

Kit components:

Reagents	100T	500T	Storage
TBA	25mg	125mg	-20°C , away from light
TBA solvent	6.76mL	35mL	-20°C
TBA Diluent	15mL	75mL	-20°C
Antioxidant	300 μ L	1.5mL	-20°C , away from light
Standard（1mM）	200 μ L	1.mL	-20°C

Storage:

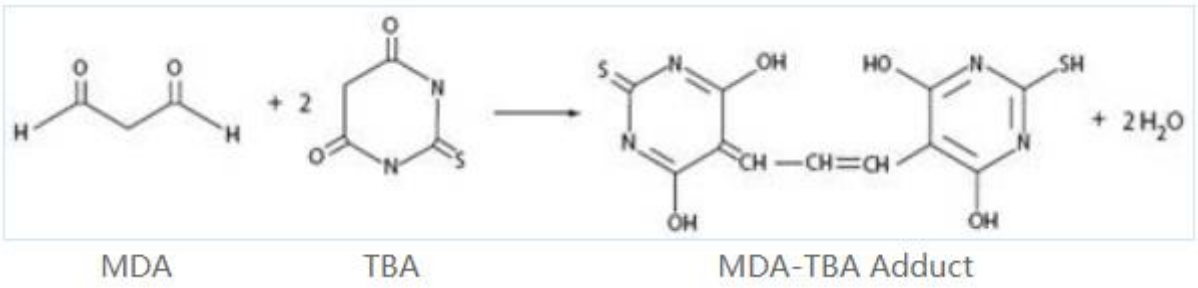
Store at -20℃ away from light for one year. TBA, TBA solvent, TBA Diluent can be stored at room temperature or 4℃ for three months.

Introduction:

Lipid Peroxidation MDA Assay Kit uses a color reaction based on the reaction of MDA and thiobarbituric acid (TBA) to produce red product. It is used to determine MDA in plasma, serum, urine, animal and plant tissue or cell lysate by colorimetry. It is widely used as a test kit to detect lipid peroxidation level.

Malondialdehyde (MDA) is a natural product of lipid oxidation. Lipid oxidation occurs when oxidative stress occurs in animal or plant cells.Some fatty acids are oxidized and gradually broken down into a range of complex compounds, including MDA. At this time, the level of lipid oxidation can be detected by detecting the level of MDA, so the determination of MDA is widely used as an indicator of lipid oxidation. MDA is also produced by some other biochemical reactions in vivo, such as thromboxane synthase, but changes in lipid oxidation levels can be observed as long as appropriate controls are set during measurement.

Malondialdehyde can react with TBA in high temperature and acidic environment to form the red MDA-TBA adduct. The corresponding reaction principle diagram is as follows:



The MDA-TBA adduct has a maximum absorption at 535nm, which can be detected by colorimetry. In addition, the MDA-TBA adduct can also be excited at 535nm to produce a maximum emission wavelength of 553nm, according to which fluorescence detection can also be performed.

Assay Protocol:

一、Sample Preparation

1. Plasma, serum, or urine samples can be directly used for MDA determination after preparation.

2. Tissues or cells may be homogenized using PBS or other cell lysates.For tissue, the proportion of tissue weight to homogenate or lysate is 10%; For cells, 0.1ml of lysate or homogenate is used per 1 million cells. After homogenization or cracking, the supernatant was obtained by centrifugation at 10,000-12,000 g for 10 minutes for subsequent determination. For some special samples, the clarified supernatant solution cannot be obtained by centrifugation, and the clarified sample solution can be filtered by a filter with a diameter of 0.2 microns. Sample preparation steps such as homogenizing or cracking should be performed in an ice bath or at 4 ℃.

3. For tissue or cell samples, the protein concentration can be determined with BCA Protein Assay Kit (K001) after the sample is prepared, so as to facilitate the subsequent calculation of MDA content in the tissue or cell per unit protein weight.

二、Kit Preparation

1. Preparation of TBA storage solution: Weigh an appropriate amount of TBA and prepare TBA storage solution with a concentration of 0.37%. For example, 18.5mg TBA is prepared with 5ml TBA solvent, or 25mg TBA is prepared with 6.76ml TBA solvent, and the final concentration is 0.37%. The TBA solvent should be completely dissolved before use and can be heated to 70 ℃ to promote dissolution. TBA storage solution is difficult to dissolve and needs to be heated to 70℃ and subjected to intense Vortex to promote dissolution. The prepared TBA storage solution is stored at room temperature and away from light for at least 3 months.

2. Preparation of MDA test solution: According to the number of samples to be measured (including control), prepare an appropriate amount of MDA test solution fresh before the detection by referring to the following table.

Number of samples	1 次	10 次	20 次	50 次
TBA Diluent	150μL	1500μL	3000μL	7500μL
TBA storage solution	50μL	500μL	1000μL	2500μL
Antioxidant	3μL	30μL	60μL	150μL

Note: The MDA test solution is difficult to dissolve and can be heated at 70 ℃ and violently Vortex to promote dissolution. It can also be treated with ultrasound to promote dissolution. The prepared MDA test solution must be used on the same day.

3. Dilution of standard products: Take appropriate amount of standard and dilute them with distilled water to 1, 2, 5, 10, 20, 50μM for subsequent production of standard curves. If the concentration of MDA in the sample is very high, the standard concentration of 100, 150, and 200μM can be increased.

三、Sample Determination

1. Add 0.1ml homogenate, lysate or PBS in a centrifuge tube or other appropriate container as a blank control, add 0.1ml of the above standard products with different concentrations for preparing standard curves, and add 0.1ml of samples for determination; Then 0.2ml of MDA detection fluid was added. You can refer to the following table to set up the detection reaction system:

	blank control	standard	sample
homogenate, lysate or PBS	0.1mL	-	-
standard	-	0.1mL	-
sample	-	-	0.1mL
MDA test solution	0.2mL	0.2mL	0.2mL

2. After mixing, heat at 100°C or in a boiling water bath for 15 minutes. When heating, be careful to avoid liquid boiling spatter. If Heat block is used for heating, press the centrifugal tube cover tightly with heavy objects; If using a boiling water bath, use a centrifuge tube with a lockable lid or a screw cap centrifuge tube, or seal the nozzle of the centrifuge tube with a Parafilm and puncture a small hole with a needle. The most convenient and accurate method of heating is to use a PCR instrument with a hot cap that can heat a 0.5ml PCR tube.

3. Cool the water bath to room temperature and centrifuge at 1000g for 10 minutes. 200 microliters of supernatant were added to the 96-well plate, and the absorbance was measured at 532nm by enzyme-labeled analyzer. If it is not convenient to determine the absorbance of 532nm, it is also possible to determine the absorbance between 530-540nm. Dual wavelength determination can be performed by setting 450nm as the reference wavelength.

4. Calculation of MDA content: For samples such as plasma, serum or urine, the MDA molar concentration can be calculated directly according to the standard curve. For cell or tissue samples, after calculating the MDA content in the sample solution, the MDA 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.

Note:

1. Aldehydes and relatively high concentrations of soluble sugars (such as 250mM sucrose) interfere with the reaction, and the products of soluble sugar and TBA color reaction are also absorbed at 532nm (the maximum absorption is 450nm).If soluble sugar interferes with the determination, dual-wavelength determination can be performed by measuring 450nm as a reference wavelength to eliminate the interference.

2. For your safety and health, please wear the lab coat and disposable gloves before the experiments.

3. This kit is for research use only.