(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Malondialdehyde (MDA) Assay Kit

Catalog No.: BC00008

Size: 100T

Please read the instructions carefully before use. 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

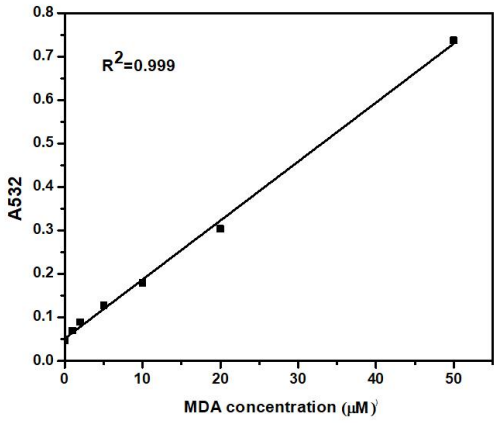
Product Name	Malondialdehyde (MDA) Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, Plasma, Urine , Tissues, Cells
Assay Type	Quantitative
Detection Instrument	Microplate reader (530-540 nm, optimal detection wavelength 532 nm. Dual wavelength determination reference wavelength 450 nm)
Range	1-200μM
Sensitivity	0.5342μM

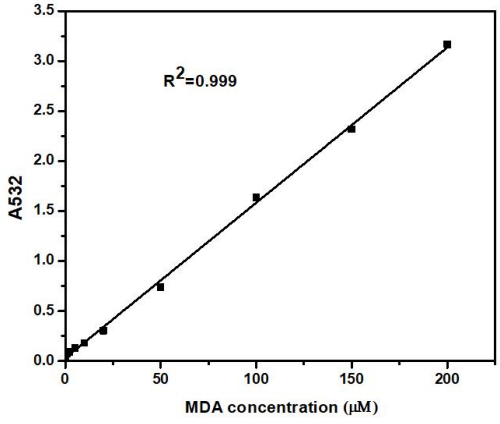
Product Introduction

Lipid oxidation refers to the process in which lipid molecules in cells or the body suffer oxidative damage, which can lead to impaired cell and tissue function and cause a series of diseases. In medical and life science research, one of the methods to measure the degree of lipid oxidation is to detect the level of lipid oxidation product malondialdehyde (MDA).

Product Features

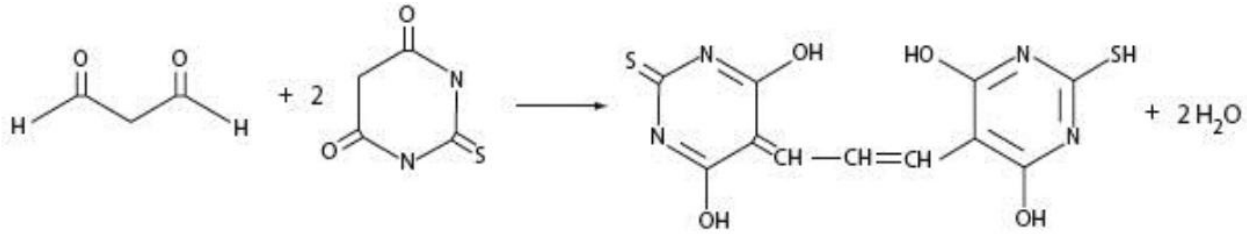
- This kit can measure MDA in the range of 1-200μM. The figure below shows the A532 readings of different concentrations of MDA standards.





Principle

This kit uses thiobarbituric acid (TBA) to react with MDA to produce a red product (MDA-TBA adduct). The MDA-TBA adduct has a maximum absorption at 532nm, which can be detected by colorimetry. In addition, the MDA-TBA adduct can also be excited at 532nm to produce a maximum emission wavelength of 532nm, which can also be used for fluorescence detection.



MDA TBA MDA-TBA Adduct

Components

Serial number	Components	Size(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 3 months.
Reagent 2	TBA preparation solution	6.76ml	-20℃, after opening the bottle can be stored at room temperature or 2-8 degrees for 3 months.
Reagent 3	TBA diluent	15ml	-20℃, after opening the bottle can be stored at room temperature or 2-8 degrees for 3 months.
Reagent 4	Antioxidants	300μl	-20℃, keep away from light.
Reagent 5	Standard (1 mM)	200μl	-20℃
Consumable 1	Microplate(96 wells)	1 board	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

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

Experimental Preparation

- Sample processing

1. Plasma, serum or urine samples can be directly used for MDA 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 lyse buffer is 10%; for cells, 0.1ml lysis buffer or homogenate is used for every 1 million cells. After homogenization or lysis, centrifuge at 10,000 x g-12,000 x g 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 the MDA detection working solution, filter it with a 0.2μm 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 BCA Protein Assay Kit (BCA Method) (BC00006) produced by EnkiLife , so as to facilitate the subsequent calculation of the MDA content in the tissue or cell per unit protein weight.

- Preparation of the assay 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, 18.5 mg of TBA is prepared with 5 ml of TBA preparation solution, or 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 70ºC to promote dissolution. The TBA stock solution is difficult to dissolve and needs to be heated to 70ºC and violently vortexed to promote dissolution. The prepared TBA stock solution should be stored at room temperature away from light and is valid for at least 3 months.
2. Preparation of MDA 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 MDA working solution just before testing.

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

Note: MDA working solution is difficult to dissolve. It can be heated to 70° C and vortexed vigorously to promote dissolution. It can also be sonicated to promote dissolution. The MDA 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 MDA working solution must be used on the same day.

3. Dilution of standard: Take an appropriate amount of standard and dilute it with distilled water to 50, 25, 12.5, 6.25, 3.125, 0μM using half-dilution method for subsequent preparation of standard curve. For example, first prepare 220μl of 50μM standard, take 110μl and add 110μl of diluent to prepare 220μl of 25μM standard, and so on. If the concentration of MDA in the sample is very high, the concentration of 100 and 200μM standard can be increased.

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; then add 0.2ml of MDA 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 1000 x g for 10 minutes at room temperature. Take 200μl 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
MDA detection working solution	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 microplate reader.

Result calculation

For samples such as plasma, serum or urine, the molar concentration of MDA can be directly calculated based on the standard curve. For cells or tissue samples, after calculating the MDA content in the sample solution, the MDA content in the original 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 MDA was detected. The MDA concentration in the sample may be too low and below the detection limit. When testing MDA in tissues or cells, please use more tissues or cells. And be careful not to dilute the sample as much as possible.
2.Since the MDA working solution is unstable, it is recommended to make a standard curve each time, or to use the same conditions for processing standards and samples.
3.This product is for Research Use Only and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.