PRODUCT INFORMATION & MANUAL

Malondialdehyde Assay Kit (Colorimetric)

NBP3-24519

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

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Malondialdehyde Assay Kit (Colorimetric)

Catalog No: NBP3-24519

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.38 nmol/mL

Detection range: 0.38-133.33 nmol/mL

Average intra-assay CV (%): 4.9

Average inter-assay CV (%): 8.0

Average recovery rate (%): 101

- 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

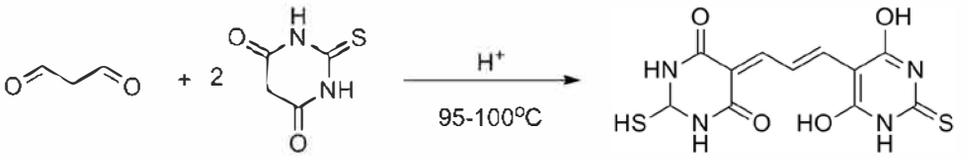
This kit can be used to measure the MDA content in serum, plasma and animal tissue samples.

▲Background

The body produce oxygen free radicals through the enzyme system and non-enzyme system, which can attack unsaturated fatty acid on biofilm and lead to lipid peroxidation and form lipid peroxide, such as aldehyde group (MDA), keto-, hydroxyl, carbonyl, etc. Oxygen free radicals cause cell damage not only by peroxidation of polyunsaturated fatty acids in biofilm, but also by decomposition products of lipid hydroperoxide. Detection of the MDA content can reflect the level of lipid peroxidation in cells and reflect level of cellular damage indirectly.

▲Detection principle

MDA in the catabolite of lipid peroxide can react with thiobarbituric acid (TBA) and produce red compound, which has a maximum absorption peak at 532 nm.



▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Clarificant	24 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Acid Reagent	12 mL × 1 vial	2-8℃ ,12 months
Reagent 3	Chromogenic Agent	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 4	10 nmol/mL Standard	5 mL × 1 vial	2-8℃ ,12 months
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

Spectrophotometer (532 nm), Micropipettor, Vortex mixer,Incubator, Centrifuge

Reagents

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), Glacial acetic acid (analytical reagent, acetic acid concentration ≥99.5%), Absolute ethanol

⚠ 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. It is recommended to fasten the glass tube mouth with preservative film and make a small hole in the film.

2. The temperature of water-bath and the time of incubation should be stabilized (95-100℃ , 40 min).

3. The supernatant for assay should not contain sediment, otherwise it will affect the OD values. It is recommended to use a pipette to take the supernatant.

Pre-assay preparation

▲Reagent preparation

1. Reagent 1 will be solidification when the weather is cold, please warm it in 37℃ water bath until the liquid turns to transparent before the experiment.
2. Reagent 2 application solution
   Dilute reagent 2 with double-distilled water at a ratio of 1.2: 34 and mix fully.
3. Reagent 3 application solution (chromogenic agent)
   Dissolve a vial of the powder with 30 mL of double-distilled water 90℃ ~100℃), then add 30 mL of glacial acetic acid, mix fully and cool to room temperature. The prepared solution can be store at 2-8℃ with shading light for 1 month.

▲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 (0.38-133.33 nmol/mL).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	1
Human plasma	1
Rat serum	1
Rat plasma	1
Mouse serum	1
Mouse plasma	1
10% Rat heart tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat brain tissue homogenate	1

Assay protocol

▲Detailed operation steps

1. Blank tube: Take A* mL of absolute ethanol into the 10 mL glass test tubes.
   Standard tube: Take A* mL of 10 nmol/mL Standard into the 10 mL glass test tubes.
   Sample tube: Take A* mL of tested Sample into numbered 10 mL glass test tubes.
   Control tube: Take A* mL of tested Sample into numbered 10 mL glass test tubes.
2. Add A* mL of reagent 1 into each tube of Step 1.
3. Add 3 mL of reagent 2 application solution into each tube of Step 2.
4. Add 1 mL of reagent 3 application solution into Blank tube, Standard tube, Sample tube, add 1 mL of 50% glacial acetic acid into Control tube.
5. Mix fully and fasten the mouth of the tube with plastic film, prick a small hole with a needle. Then incubate the tubes at 95-100℃ for 40 min.
6. Cool the tubes to room temperature with running water, centrifuge the tubes at 3100 g for 10 min.
7. Take 3 mL the supernatant of each tube. Set the spectrophotometer to zero with double distilled water and measure the OD value at 532 nm with 1 cm optical path cuvette (the precipitation cannot be added to the cuvette).

Note:

1. A* represents the volume of sample, standard, absolute ethanol and reagent 1, they are equal.
   For example, the sampling volume is 0.1 mL, the volume of standard, absolute ethanol and reagent 1 are 0.1 mL for each. If the sampling volume is 0.2 mL, the volume of standard, absolute ethanol and reagent 1 are 0.2 mL for each.
2. In general, 1~2 tubes of standard and blank are established for each batch. If the serum (plasma) samples are no hemolysis or lipidemia, control tube can be remove, just need to establish blank tube.
3. Reference sampling volume:
   Serum (plasma): 0.1~0.2 mL, Low density lipoprotein suspension: 0.1~0.2 mL,
   Liver tissue, myocardium, muscle tissue: 5% or 10% tissue homogenate, 0.1~0.2 mL.

▲Summary operation table

	Blank tube	Standard tube	Sample tube	Control tube
Absolute ethanol (mL)	A*			
10 nmol/mL Standard (mL)		A*		
Samples (mL)			A*	A*
Reagent 1 (mL)	A*	A*	A*	A*
Reagent 2 application solution (mL)	3.0	3.0	3.0	3.0
Reagent 3 application solution (mL)	1.0	1.0	1.0	
50% glacial acetic acid (mL)				1.0
Mix fully and fasten the mouth of the tube, prick a small hole. Then incubate the tubes at 95-100℃ for 40 min. Cool the tubes to room temperature, centrifuge the tubes at 3100 g for 10 min. Take 3 mL the supernatant of each tube. Set the spectrophotometer to zero.				

▲Calculation

1. For serum and plasma

$$\text{MDA content(nmol/mL)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. For tissue sample:

$$\text{MDA content(nmol/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

Note:

ΔA_1: $OD_{Sample} - OD_{Control}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: The concentration of standard, 10 nmol/mL.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, mgprot/mL.

Appendix I Data

▲Example analysis

Take 0.1 mL of human serum and carry the assay according to the operation table.

The results are as follows:

the average OD value of the sample is 0.069, the average OD value of the blank is 0.002, the average OD value of the standard is 0.060, and the calculation result is:

$$\text{MDA content(nmol/mL)} = \frac{0.069-0.002}{0.060-0.002} \times 10 = 11.55 \text{ nmol/mL}$$

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 1000-2000 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.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ to remove blood cells. 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 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. 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).

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.)

Appendix III References

1. Del R D, Stewart A J, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition Metabolism & Cardiovascular Diseas, 2005, 15(4): 316-328.
2. Gaschler M M, Stockwell B R. Lipid peroxidation in cell death. Biochemical & Biophysical Research Communications, 2017, 482(3): 419-425.
3. Niki E. Biomarkers of lipid peroxidation in clinical material. Biochim Biophys Acta, 2014, 1840(2): 809-817.
4. Draper H H, Hadley M. Malondialdehyde Determination as Index of Lipid Peroxidation. Methods Enzymol, 1990, 186(186): 421-431.
5. Nielsen F, BO B M, Nielsen J B, et al. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clinical Chemistry, 1997, 43(7): 1209-1214.