PRODUCT INFORMATION & MANUAL

Malondialdehyde Assay Kit (Colorimetric)

NBP3-24518

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

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Malondialdehyde Assay Kit
(Colorimetric)

Catalog No: NBP3-24518

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 1.13 μmol/L

Detection range: 2.92-40 μmol/L

Average intra-assay CV (%): 4.1

Average inter-assay CV (%): 7.2

Average recovery rate (%): 97.8

▲ 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

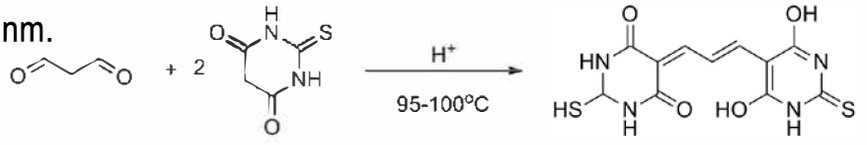
This kit can be used to measure the Malondialdehyde content in serum, plasma, tissue and other samples.

▲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 (Malondialdehyde), 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 Malondialdehyde content can reflect the level of lipid peroxidation in cells and reflect level of cellular damage indirectly.

▲Detection principle

Malondialdehyde 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	3 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Acid Reagent	4 mL × 1 vial	2-8℃ ,12 months
Reagent 3	Chromogenic Agent	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	50 μmol/L Standard	5 mL × 1 vial	2-8℃ ,12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader (530-540 nm), Micropipettor, Vortex mixer, Incubator, Centrifuge

Reagents

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), Acetic acid, 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. In the incubation of 100℃ water bath, the EP tube should not be closed directly. It is recommended to fasten the tube mouth with fresh-keeping 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 colorimetric measurement 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 frozen when store at 2-8℃ for a long time, please warm it in 37℃ water-bath until clear.
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:
   Dissolve the powder with 14 mL of double-distilled water (90~100℃) fully , then add 14 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. (Glacial acetic acid, analytical reagent, acetic acid concentration ≥99.5%. This reagent should be self-prepared.)
4. Preparation of 50% acetic acid

   Add 8 mL of glacial acetic acid into 8 mL of double distilled water slowlly and mix fully. Stand at room temperature for detection (Note: Glacial acetic acid with high concentrations , please add slowly during the dilution process)

▲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 formal experiment and the detection range (2.92-40 μmol/L).

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

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 50 μmol/L Standard with absolute ethyl alcohol to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	50 μmol/L Standard (μL)	Absolute ethyl alcohol (μL)
A	0	0	100
B	5	10	90
C	10	20	80
D	15	30	70
E	20	40	60
F	25	50	50
G	30	60	40
H	40	80	20

2. The measurement of samples

(1) Standard tube: Take 0.02 mL of standard solution with different concentrations into numbered 1.5 mL EP tubes.

Sample tube: Take 0.02 mL of sample into numbered 1.5 mL EP tubes.

Control tube: Take 0.02 mL of sample into numbered 1.5 mL EP tubes.

(2) Add 0.02 mL of reagent 1 into each tube of Step (1).

(3) Add 0.6 mL of reagent 2 application solution into each tube of Step (2).

(4) Add 0.2 mL of reagent 3 application solution into standard tubes and sample tubes, add 0.2 mL of 50% acetic acid to the control tubes.

(5) Fasten the tube mouth with fresh-keeping film, mix fully, and make a small hole in the film. Then incubate the tubes at 100℃ for 40 min.

(6) Cool the tubes to room temperature with running water, centrifuge the tubes at 9569 g for 10 min.

(7) Take 0.25 mL the supernatant of each tube to the microplate with a micropipette (the precipitation cannot be added to the microplate).

(8) Measure the OD value at 532 nm with microplate reader.

Note: In general, the serum (plasma) samples are no hemolysis or lipidemia, control tube can be removed, just need to establish blank (the concentration of standard is 0 μmol/L) tube.

▲Summary operation table

	Standard tube	Sample tube	Control tube
Standard solution with different concentrations (mL)	0.02		
Sample (mL)		0.02	0.02
Reagent 1 (mL)	0.02	0.02	0.02
Reagent 2 application solution (mL)	0.6	0.6	0.6
Reagent 3 application solution (mL)	0.2	0.2	
50% acetic acid (mL)			0.2
Mix fully, fasten the tube mouth with fresh-keeping film, mix fully, and make a small hole in the film. Then incubate the tubes at 100℃ for 40 min. Cool the tubes to room temperature, centrifuge the tubes at 9569 g for 10 min. Take 0.25 mL the supernatant of each tube. Measure the OD value at 532 nm.			

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

1. Serum (plasma) samples:

$$\text{MDA (μmol/L)} = (\Delta A\text{-}b) \div a \times f$$

2. Tissue samples:

$$\text{MDA (μmol/gprot)} = (\Delta A\text{-}b) \div a \times f \div C_{pr}$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, gprot/L.

ΔA: $OD_{Sample} - OD_{Blank}$ or $OD_{Sample} - OD_{Control}$.

Appendix I Data

▲Example analysis

Take 0.02 mL of 10% rat liver tissue homogenate and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.0057x-0.0015, the average OD value of the sample is 0.075, the average OD value of the blank is 0.041, the concentration of protein in sample is 12.89 gprot/L, and the calculation result is:

MDA (μmol/gprot) =(0.075-0.041+0.0015)÷0.0057÷12.89
=0.48 μmol/gprot

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 (Heparin is used as 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 (E-BC-K318-M). 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.