PRODUCT INFORMATION & MANUAL

Malondialdehyde Assay Kit (Colorimetric)

NBP3-24515

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

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Malondialdehyde Assay Kit (Colorimetric)

Catalog No: NBP3-24515

Method: Colorimetric method

Specification: 96T (Can detect 92 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.29 nmol/mL

Detection range: 0.29-100 nmol/mL

Average intra-assay CV (%): 3.3

Average inter-assay CV (%): 3.5

Average recovery rate (%): 95

- ▲ 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

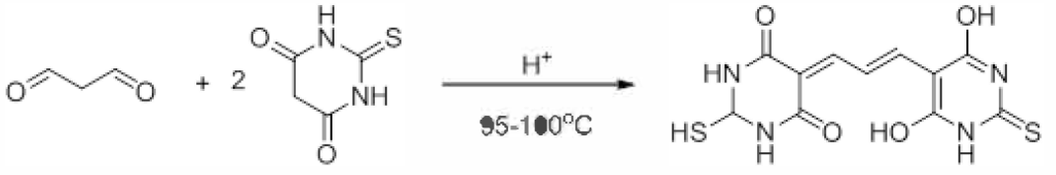
This kit can be used to measure the malondialdehyde (MDA) content in cell samples.

▲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	6 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Acid Reagent	6 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	30 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	10 nmol/mL Standard	5 mL × 1 vial	2-8℃ , 12 months
Reagent 5	Extracting Solution	60 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, Centrifuge, Incubator, Water bath, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, 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. Water-bath temperature (95-100℃) and incubation time (40 min) should be stabilized. Cool the tubes with running water immediately once the incubation finished.
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.
4. Accurately take 250 μL reaction solution into the 96 wells microplate and without bubble.
5. If solid is precipitated in reagent3, heat it in 80℃ with water bath until dissolved.

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. Preparation of reagent 2 application solution:
   Dilute the reagent 2 with double distilled water at a ratio of 1.2: 34 and mix fully. The prepared solution can be stored at 2-8℃ for 3 months.
3. Preparation of working solution:
   Mix the reagent 1, reagent 2 application solution, reagent 3 at a ratio of 0.2: 3: 1 fully. Prepare the fresh solution before use.

▲Sample preparation

Cell samples

Collect the cells into a centrifuge tube, add reagent 5 at a ratio of cell number ($3*10^6$): reagent 5 (μL) =1： 300-500 , mix fully for 2 min, then treat the cell with sonication (90W, 4s/time, interval for 2s, the total time is 10 min) or homogenization. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲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 (0.29-100 nmol/mL).

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

Sample type	Dilution factor
A549 cells	1
HepG2 cells	1
293T cells	1

Note: The diluent is reagent 5 .

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

Note: A, blank well; B, standard wells; S1-S92, sample wells.

▲Detailed operation steps

1. Blank tube:Take 0.1 mL of absolute ethanol (self-prepared) to the 1.5 mL EP tubes
   Standard tube: Take 0.1 mL of 10 nmol/mL standard to the 1.5 mL EP tubes.
   Sample tube:Take 0.1 mL of sample to the 1.5 mL EP tubes.
2. Add 1 mL of working solution into the wells of Step 1.
3. Mix fully with a vortex mixer. Tighten the tubes with preservative film and make a hole in the film. Incubate the tubes in 100℃ water bath for 40 min.
4. Cool the tubes to room temperature with running water. Centrifuge at 1078 g for 10 min.
5. Take 250 μL of supernatant to microplate and measure the OD value at 532 nm with microplate reader.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Absolute ethanol (self-prepared) (mL)	0.1		
10 nmol/mL standard (mL)		0.1	
Sample (mL)			0.1
Working solution (mL)	1	1	1
Mix fully. Tighten the tubes with preservative film and make a hole in the film. Incubate the tubes in 100℃ water bath for 40 min. Cool the tubes to room temperature. Centrifuge at 1078 g for 10 min. Take 250 μL of supernatant to microplate and measure the OD value.			

▲Calculation

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

ΔA_1: $OD_{Sample} - OD_{Blank.}$

ΔA_2: $OD_{Standard} - OD_{Blank.}$

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

f: Dilution factor of sample before test.

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

Appendix I Data

▲Example analysis

Take 0.1 mL of HepG2 cell homogenate and carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.068, the average OD value of the blank is 0.043, the average OD value of the standard is 0.211, the concentration of protein in sample is 3.38 mgprot/mL, and the calculation result is:

$$\text{MDA (nmol/mgprot)} = \frac{0.068-0.043}{0.211-0.043} \times 10 \div 3.38 = 0.44 \text{ nmol/mgprot}$$

Appendix II 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.