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

Catalog No：E-BC-K028-M

Specification：500 Assays(496 samples)

Measuring instrument：Microplate reader（530-540 nm）

Detection range：0.29-100 nmol/mL

Elabscience® Malondialdehyde (MDA) Colorimetric Assay Kit (Cell Samples)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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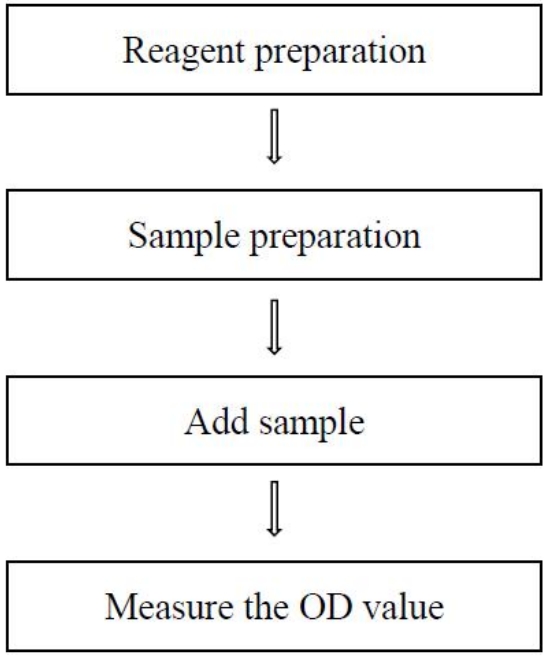
Assay summary

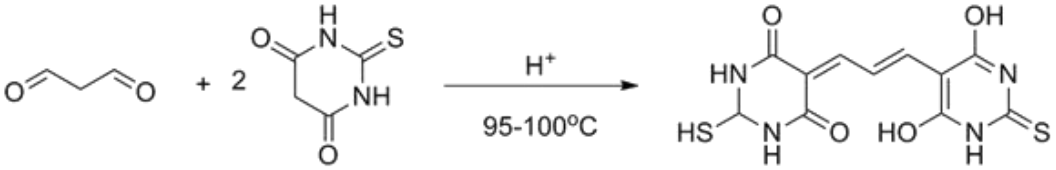


Intended use

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

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	Size (500 Assays)	Storage
Reagent 1	Clarificant	30 mL × 1 vial	2-8℃, 12 months
Reagent 2	Acid Reagent	30 mL × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	50 mL × 3 vials	2-8℃, 12 months shading light
Reagent 4	10 nmol/mL Standard	5 mL × 1 vial	2-8℃, 12 months
Reagent 5	Extracting Solution	30 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Absolute ethanol

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② Clarificant will be frozen when store at 2-8℃ for a long time, please warm it in 37℃ water-bath until clear.

③ The preparation of acid application solution：
Before testing, please prepare sufficient acid application solution according to the test wells. For example, prepare 880 μL of acid application solution (mix well 30 μL of acid reagent and 850 μL of double distilled water). Store at 2-8℃ for 3 months.

④ The preparation of working solution：
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 1050 μL of working solution (mix well 50 μL of clarificant, 750 μL of acid application solution t and 250 μL of chromogenic agent).

⑤ The preparation of extraction working solution：
Before testing, please prepare sufficient extraction working solution according to the test wells. For example, prepare 500 μL of extraction working solution (mix well 50 μL of extraction solution and 450 μL of double distilled water). The prepared extraction working solution can be stored at 2-8℃ for 3 months.

Sample preparation

① Sample preparation

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 3×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 3×10^6 cells in 500 μL extraction working solution with a ultrasonic cell disruptor at 4℃, and keep it on ice for detection.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material, and collect supernatant to determine the protein concentration (E-BC-K318-M).

② Dilution of sample

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 extraction working solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

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 chromogenic agent, heat it in 80℃ with water bath until dissolved.

Operating steps

① Blank tube: add 0.1 mL of absolute ethanol (self-prepared) to the 1.5 mL EP tubes.

Standard tube: add 0.1 mL of 10 nmol/mL standard to the 1.5 mL EP tubes.

Sample tube: 0.1 mL of sample to the 1.5 mL EP tubes.

② Add 1 mL of working solution into each tube.

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 250μL the supernatant of each tube to the microplate with a micropipette.

⑥ Measure the OD values of each well at 532 nm with microplate reader.

Calculation

The sample:

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

[Note]

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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three HepG2 cell samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mL)	2.60	34.50	88.50
%CV	3.6	3.2	3.1

Intra-assay Precision

Three HepG2 cell samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean(nmol/mL)	2.60	34.50	88.50
%CV	3.1	3.8	3.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (nmol/mL)	12	49	91
Observed Conc. (nmol/mL)	11.2	48.0	85.5
Recovery rate (%)	93	98	94

Sensitivity

The analytical sensitivity of the assay is 0.29 nmol/mL MDA. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding

concentration.

Appendix Π Example Analysis

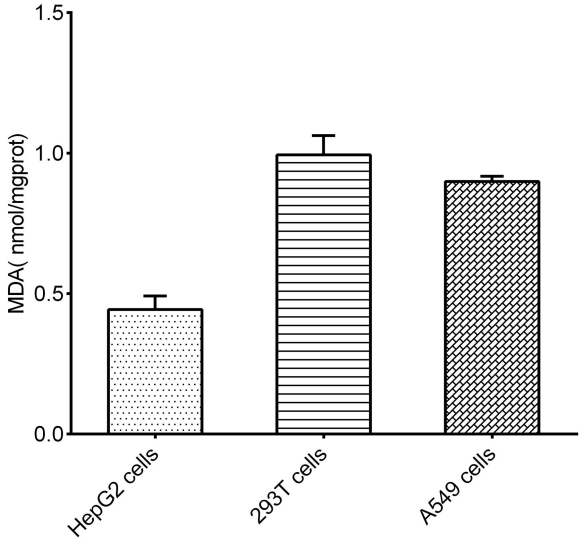
Example analysis：

Take 0.1 mL of HepG2 cell homogenate and carry the assay according to the operation steps. 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:

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

Detect HepG2 cells (the concentration of protein is 3.38 mgprot/mL), 293T cells (the concentration of protein is 2.73 mgprot/mL) and A549 cells (the concentration of protein is 0.67 mgprot/mL) according to the protocol, the result is as follows：



Appendix III Publications

1. Qiu C, Tang C, Tang Y, et al. RGS5+ lymphatic endothelial cells facilitate metastasis and acquired drug resistance of breast cancer through oxidative stress-sensing mechanism[J]. Drug Resistance Updates, 2024, 77: 101149.
2. Wang X, Wang J, Liu S, et al. Sterilization mechanism and nanotoxicity of visible light-driven defective carbon nitride and UV-excited TiO2[J]. Journal of Hazardous Materials, 2024, 461: 132109.
3. Wu Z, Chen K, Mo W, et al. Multimodal enhancement of ferroptosis for synergistic cascade colorectal cancer therapy[J]. Chemical Engineering Journal, 2024, 498: 155048.
4. Zhang H, Feng Y, Si Y, et al. Shank3 ameliorates neuronal injury after cerebral ischemia/reperfusion via inhibiting oxidative stress and inflammation[J]. Redox Biology, 2024, 69: 102983.
5. Zhang T, Wang S, Hua D, et al. Identification of ZIP8-induced ferroptosis as a major type of cell death in monocytes under sepsis conditions[J]. Redox Biology, 2024, 69: 102985.
6. Tian Y, Guo Z, et al. 17 β -oestradiol inhibits ferroptosis in the hippocampus by upregulating DHODH and further improves memory decline after ovariectomy[J]. Redox Biology, 2023, 62: 102708.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.