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

Catalog No：E-BC-K814-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader(530-540 nm)

Detection range：0.19-100.00 nmol/mL

Elabscience® Enhanced Cell Malondialdehyde (MDA) Colorimetric Assay Kit

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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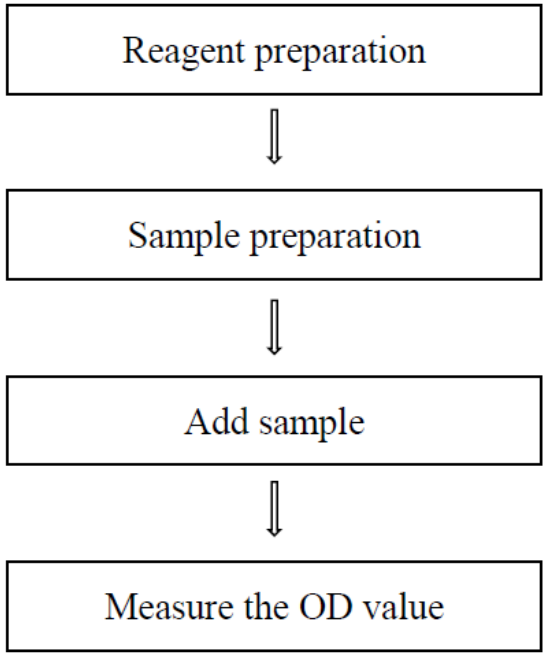
Assay summary

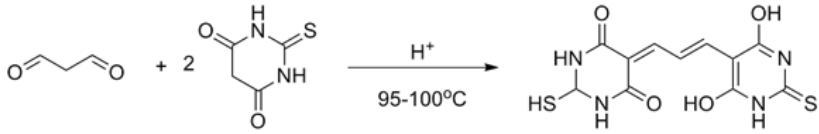


Intended use

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

Detection principle

Malondialdehyde (MDA) in the degradation products of peroxidized lipids can react with thiobarbituric acid (TBA) under high temperature and acidic conditions to produce the red-brown product 3,5,5 '-trimethyloxazole-2, 4-dione (trimethine), and this substance has the maximum absorption peak at 532 nm.



Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Clarificant	3 mL ×1 vial	6 mL ×1 vial	2-8℃, 12 months
Reagent 2	Acid Reagent A	1 mL ×1 vial	1 mL ×1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	Powder ×1 vial	Powder ×2 vials	2-8℃, 12 months shading light
Reagent 4	10 nmol/mL Standard	5 mL ×1 vial	5 mL ×1 vial	2-8℃, 12 months
Reagent 5	Lysis Solution	30 mL ×1 vial	60 mL ×1 vial	2-8℃, 12 months
Reagent 6	Diluent Solution	1.8 mL ×1 vial	3.6 mL ×1 vial	2-8℃, 12 months shading light
Reagent 7	Acid Reagent B	6 mL ×1 vial	12 mL ×1 vial	2-8℃, 12 months
	Microplate	48 wells	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. 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, optimum wavelength: 532 nm), Incubator (37 ℃), Water bath (100 ℃)

Reagent preparation

① Equilibrate all reagents to 25 ℃ before use.

② The clarificant will be frozen when store at 2-8℃, please warm it in 37℃.

③ The preparation of acid application solution:
Before testing, please prepare sufficient acid application solution. For example, prepare 100 μL of acid application solution (mix well 5 μL of acid reagent A and 95 μL of double distilled water). The acid application solution should be prepared on spot and used up within 8 hours.

④ The preparation of chromogenic application solution：
Dissolve one vial of chromogenic agent with 1.5 mL of diluent solution, ultrasonic oscillation to dissolve. Store at -20 ℃ for 2 months.

⑤ The lysis solution will be precipitated when store at 2-8℃, please warm it in 37℃.

⑥ The diluent solution will be frozen when store at 2-8℃, please warm it in 37℃ until clear.

⑦ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 515 μL of working solution (mix well 20 μL of clarificant, 300 μL of acid application solution, 25 μL of chromogenic application solution, 70 μL of double distilled water and 100 μL of acid reagent B). The working solution should be prepared on spot and used up within 2 hours.

Sample preparation

① Sample preparation

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

② Lyse 2×10^6 cells with 500 μL lysis solution. Mix well and place on the ice box and lyse for 3 min.

③ 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
2×10^6 A549 cells	1
2×10^6 HepG2 cells	1
2×10^6 293T cells	1

Note: The diluent is lysis solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Bubble formation was avoided during the lyse of cells with lysis solution, and at the same time there was no macroscopic flocculent in the cell suspension.

② The temperature should be controlled at 95-100℃ for 40 min of water bath reaction.

③ Avoid bubbles when adding supernatant to microplate.

Operating steps

① Blank tube: add 0.1 mL of lysis solution 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: add 0.1 mL of sample to the 1.5 mL EP tubes.

② Add 0.5 mL of working solution into each tube.

③ 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.

④ Cool the tubes to room temperature with running water. Centrifuge at 1100×g for 10 min.

⑤ Add 0.25 mL 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.

Note: If you do not use preservative film, you can first use a syringe needle to prick a small hole in the EP tube cover to prevent the tube cover from breaking open during the water bath.

Calculation

The cell sample:

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

[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)	2.50	5.00	7.50
%CV	3.3	3.0	2.1

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)	2.50	5.00	7.50
%CV	3.5	2.8	2.3

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 98.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (nmol/mL)	2.50	5.00	7.50
Observed Conc. (nmol/mL)	2.38	4.95	7.60
Recovery rate (%)	95	99	101

Sensitivity

The analytical sensitivity of the assay is 0.19 nmol/mL. 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 II Example Analysis

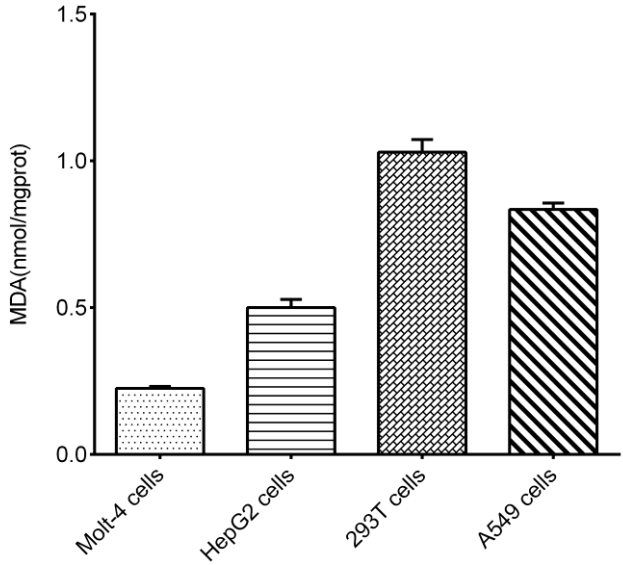
Example analysis:

Take 0.1 mL of Molt-4 cell homogenate and carry the assay according to the operation steps. The results are as follows:

The OD value of the blank tube is 0.039, the OD value of the standard tube is 0.324, the OD value of the sample tube is 0.054, the concentration of protein in sample is 2.28 mgprot/mL, and the calculation result is:

$$\text{MDA (nmol/mgprot)} = \frac{0.054 - 0.039}{0.324 - 0.039} \times 10 \div 2.28 = 0.23 \text{ nmol/mgprot}$$

Detect 2×10^6 Molt-4 cells (the concentration of protein is 2.28 mgprot/mL), 2×10^6 HepG2 cells (the concentration of protein is 3.38 mgprot/mL), 2×10^6 293T cells (the concentration of protein is 2.73 mgprot/mL) and 2×10^6 A549 cells (the concentration of protein is 0.67 mgprot/mL) according to the protocol, the result is as follows:



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.