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

Catalog No：E-BC-K025-S

Specification：50 Assays (46 samples)/ 100 Assays (96 samples)

Measuring instrument：Spectrophotometer（532 nm）

Detection range：0.38-133.33 nmol/mL

Elabscience® Malondialdehyde (MDA) Colorimetric Assay Kit (TBA Method)

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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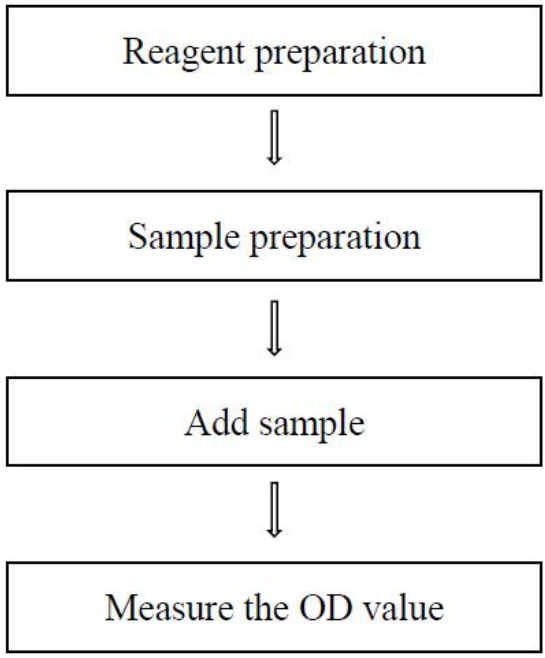
Assay summary



Intended use

This kit can be used to measure the MDA content in serum, plasma and animal tissue 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 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Clarificant	12 mL × 1 vial	24 mL × 1 vial	2-8℃, 12 months
Reagent 2	Acid Reagent	12 mL × 1 vial	12 mL × 1 vial	2-8℃, 12months
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

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:

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

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② Clarificant will be solidification when the weather is cold, please warm it in 37℃ water bath until the liquid turns to transparent before the experiment.

③ The preparation of acid application solution：
For each tube, prepare 3.0 mL of acid application solution (mix well 102.3 uL of acid reagent and 2897.7 uL of double distilled water).

④ The preparation of chromogenic application solution：
Dissolve the powder with 30 mL of double distilled water (90~100℃) fully, then add 30 mL of glacial acetic acid, mix fully and cool to room temperature. Store at 4℃ for 1 month protected from light.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (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
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). 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 The temperature (95-100℃) and the time (40 min) of incubation should be stabilized.

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.

Operating steps

① Blank tube: add A* mL of absolute ethanol into the 10 mL glass test tubes.

Standard tube: add A* mL of 10 nmol/mL standard into the 10 mL glass test tubes.

Sample tube: add A* mL of tested sample into numbered 10 mL glass test tubes.

Control tube: add A* mL of tested sample into numbered 10 mL glass test tubes.

② Add A* mL of clarificant into each tube.

③ Add 3 mL of acid reagent application solution into each tube.

④ Add 1 mL of chromogenic application solution into blank tube, standard tube and sample tube, add 1 mL of 50% acetic acid to the control tubes.

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 clarificant, they are equal. For example, the sampling volume is 0.1 mL, the volume of standard, absolute ethanol and clarificant are 0.1 mL for each. If the sampling volume is 0.2 mL, the volume of standard, absolute ethanol and clarificant 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.

Calculation

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample:

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

[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 test.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	1.20	35.60	102.50
%CV	5.3	4.8	4.6

Intra-assay Precision

Three human serum 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)	1.20	35.60	102.50
%CV	7.8	8.2	8.0

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 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (nmol/mL)	27.5	88.4	112.3
Observed Conc. (nmol/mL)	27.2	90.2	114.5
Recovery rate (%)	99	102	102

Sensitivity

The analytical sensitivity of the assay is 0.38 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

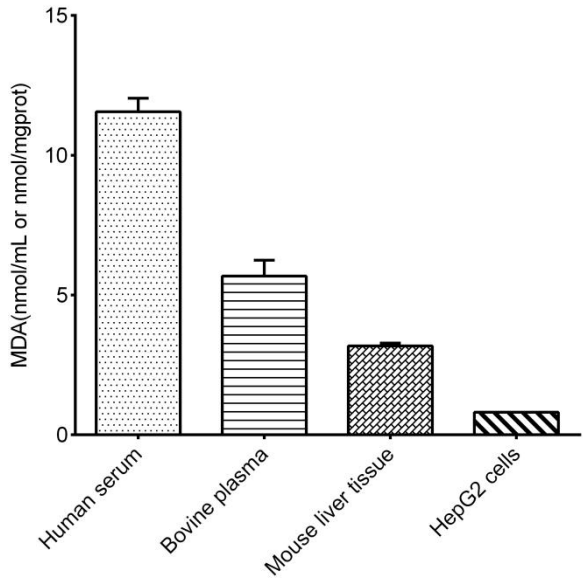
Example analysis：

Take 0.1 mL of 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:

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

Detect human serum (A*=0.1 mL), bovine plasma (A*=0.1 mL), 5% mouse liver tissue homogenate (the concentration of protein in sample is 5.61 mgprot/mL, A*=0.1 mL) and HepG2 cells (the concentration of protein in sample is 1.11 mgprot/mL, A*=0.2 mL) according to the protocol, the result is as follows：



Appendix III Publications

1. Feng R, Chen L, Yang M. Aluminum-induced oxidative stress promotes changes in the structure of the gut microbiota and liver deficiency[J]. Heliyon, 2024, 10(16).
2. Chen Z, Liu T, Luan J. Oral Administration of Lutein Improves Fat Graft Survival by Alleviating Oxidative Stress in Mice[J]. Aesthetic Surgery Journal, 2024, 44(12): NP906-NP921.
3. Al-Medhtiy M H, Mohammed M T, M. Raouf M M H, et al. A triterpenoid (corosolic acid) ameliorated AOM-mediated aberrant crypt foci in rats: modulation of Bax/PCNA, antioxidant and inflammatory mechanisms[J]. Journal of Molecular Histology, 2024, 55(5): 765-783.
4. Wang T, Yu L, Zheng J, et al. Berberine inhibits ferroptosis and stabilizes atherosclerotic plaque through NRF2/SLC7A11/GPX4 pathway[J]. Chinese Journal of Integrative Medicine, 2024, 30(10): 906-916.
5. Wang H, Li W. Puerarin alleviates the high glucose-induced oxidative stress via the RAGE/PKC/NOX4 axis in renal mesangial cells[J]. The Journal of Toxicological Sciences, 2024, 49(11): 497-507.
6. Baldea I, Moldovan R, Nagy A L, et al. Ketoconazole-Fumaric Acid Pharmaceutical Cocrystal: From Formulation Design for Bioavailability Improvement to Biocompatibility Testing and Antifungal Efficacy Evaluation[J]. International Journal of Molecular Sciences, 2024, 25(24): 13346.

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.