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

Catalog No：E-BC-K025-M

Specification：48T(32 samples)/96T(80 samples)/96T×5(464 samples)

Measuring instrument：Microplate reader（530-540 nm）

Detection range：2.92-40 μmol/L

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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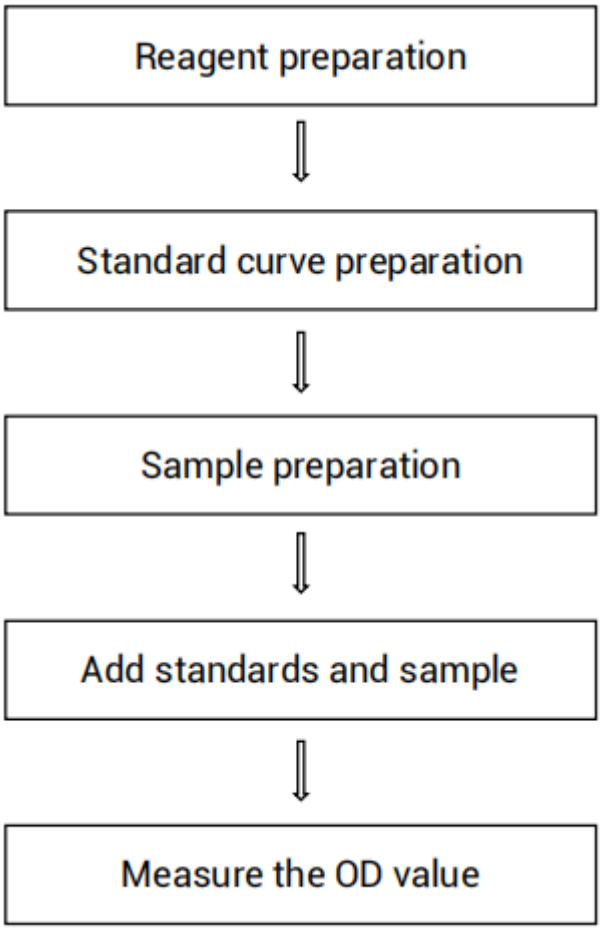
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Assay summary



Intended use

This kit can be used to measure the MDA content in serum, plasma and 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(48 T)	Size 2(96 T)	Size 3 (96 T× 5)	Storage
Reagent 1	Clarificant	3 mL×1 vial	3 mL×1 vial	15 mL×1 vial	2-8°C, 12 months
Reagent 2	Acid Reagent	2 mL×1 vial	4 mL×1 vial	20 mL×1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent	Powder×1 vial	Powder×1 vial	Powder×5 vials	2-8°C, 12 months, shading light
Reagent 4	50 μmol/L Standard	5 mL×1 vial	5 mL×1 vial	12 mL×2 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	96 wells×5	No requirement
	Plate Sealer	2 pieces		7 pieces	
	Sample Layout Sheet	1 piece		7 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), Micropipettor, Vortex mixer, Incubator, Centrifuge, Magnetic Stirrers

Reagents:

Distilled or deionized water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), Acetic acid, Absolute ethanol

Reagent preparation

① Equilibrate other reagents to room temperature before use.

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

③ The preparation of acid application solution：
Before testing, please prepare sufficient acid application solution according to the test tubes. For example, prepare 616 μL of acid application solution (mix well mix well 21 μL of acid reagent and 595 μL of distilled or deionized water).

④ The preparation of chromogenic application solution：
Dissolve chromogenic agent with 14 mL of distilled or deionized water(90~100ºC) fully, then add 14 mL of glacial acetic acid, mix fully and cool to room temperature. The prepared solution can be store at 4ºC with shading light for 1 month. (Glacial acetic acid, analytical reagent, acetic acid concentration ≥99.5%. This reagent should be self-prepared.).

⑤ The preparation of 50% acetic acid：
For each tube, prepare 0.2 mL of 50% acetic acid (mix well 100 μL of glacial acetic acid and 100 μL of distilled or deionized water. Glacial acetic acid should be added slowly during dilution.).

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 50 μmol/L standard solution with absolute ethyl alcohol to a series of concentrations. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**40**
50 μmol/L standard (μL)	0	10	20	30	40	50	60	80
Absolute ethyl alcohol (μL)	100	90	80	70	60	50	40	20

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 -80ºC 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 4ºC.

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

⑤Meanwhile, 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 In the incubation of 100°C 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°C, 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.

Operating steps

① Standard tube: add 0.02 mL of standard with different concentrations into numbered 1.5 mL EP tubes.
Sample tube: add 0.02 mL of sample into numbered 1.5 mL EP tubes.
Control tube: add 0.02 mL of sample into numbered 1.5 mL EP tubes.

② Add 0.02 mL of clarificant into each tube.

③ Add 0.6 mL of acid application solution into each tube.

④ Add 0.2 mL of chromogenic 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 100ºC 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 values of each well 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

$$MDA\ (\mu mol/L) = (\Delta A - b) \div a \times f$$

2. Tissue sample:

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

[Note]

f: Dilution factor of sample before test.

Cpr: Concentration of protein in sample, gprot/L

ΔA: Absolute OD, $OD_{Sample} - OD_{Blank}$.

(Note:The ΔA value of hemolysis or lipidemia serum (plasma) sample:OD_{Sample}-$OD_{Control}$)

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 (μmol/L)	4.60	25.30	32.50
%CV	4.4	4.0	3.9

Inter-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 (μmol/L)	4.60	25.30	32.50
%CV	8.0	6.9	6.7

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 97.8%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	12	18	29
Observed Conc. (μmol/L)	11.6	17.8	28.3
Recovery rate (%)	96.9	99	97.5

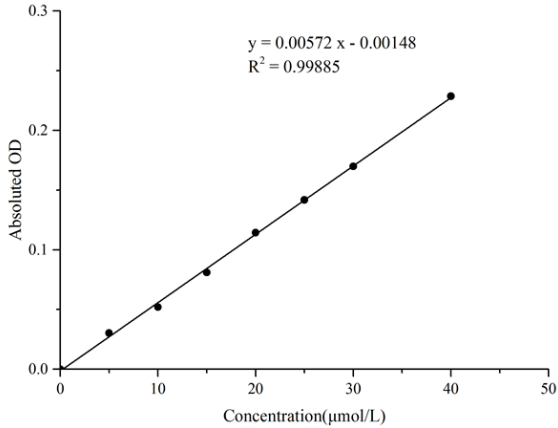
Sensitivity

The analytical sensitivity of the assay is 1.13 μmol/L 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**5**	**10**	**10**	**20**	**25**	**30**	**40**
Average OD	0.041	0.071	0.093	0.122	0.155	0.182	0.210	0.269
Absoluted OD	0	0.030	0.052	0.081	0.114	0.142	0.170	0.229



Appendix II Example Analysis

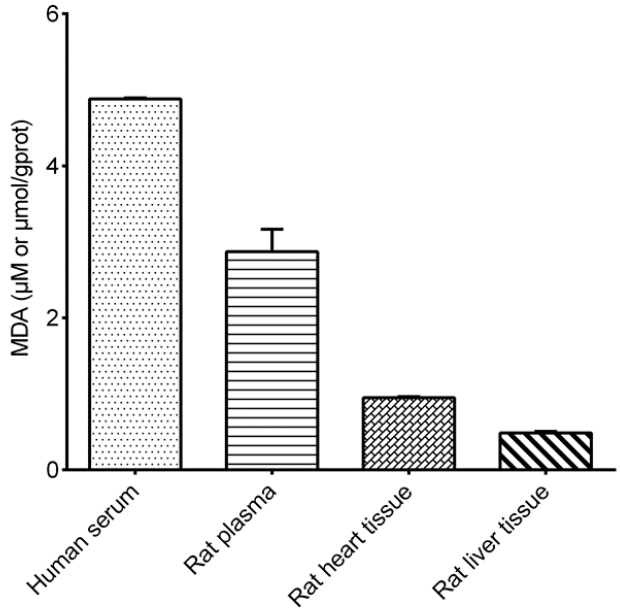
Example analysis：

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 = 0.0057 x – 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

Detect human serum, rat plasma, 10% rat heart tissue homogenate (the concentration of protein is 6.21 gprot/L) and 10% rat liver tissue homogenate (the concentration of protein is 12.89 gprot/L) according to the protocol, the result is as follows：



Appendix III Publications

1. Liu Y , Wang L , Liu Z ,et al.Durable Immunomodulatory Nanofiber Niche for the Functional Remodeling of Cardiovascular Tissue[J].ACS Nano, 2024, 18(1):21.DOI:10.1021/acsnano.3c09692.
2. Liang L , Peng W , Qin A ,et al.Intracellularly Synthesized Artificial Exosome Treats Acute Lung Injury[J].ACS Nano, 2024, 18(32):15.DOI:10.1021/acsnano.4c01900.
3. Wang Y, Liang X, Andrikopoulos N, et al. Remediation of Metal Oxide Nanotoxicity with a Functional Amyloid[J]. Advanced Science, 2024, 11(23): 2310314.
4. Xu W , B Y Y ,Yan TianCheng ChengYing ChenLianjie ZengYuan YuanDandan LiLiping ZhengTao Luo.Oral exposure to polystyrene nanoplastics reduced male fertility and even caused male infertility by inducing testicular and sperm toxicities in mice[J].Journal of Hazardous Materials, 2023, 454(Jul.15):131470.1-131470.15.DOI:10.1016/j.jhazmat. 2023.131470.
5. Liu Z, Bian Q, Wang D. Exposure to 6-PPD quinone causes ferroptosis activation associated with induction of reproductive toxicity in Caenorhabditis elegans[J]. Journal of Hazardous Materials, 2024, 471: 134356.
6. Zhao Y , Yin W , Yang Z ,et al.Nanotechnology-enabled M2 macrophage polarization and ferroptosis inhibition for targeted inflammatory bowel disease treatment[J].Journal of Controlled Release, 2024, 367:339-353.DOI:10.1016/j.jconrel.2024.01.051.

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.