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Lieferung & Zahlungsart

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Zuschläge

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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

Catalog No：E-BC-F007

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=520 nm/550 nm).

Detection range：0.04-10 μmol/L

Elabscience® Malondialdehyde (MDA)

Fluorometric 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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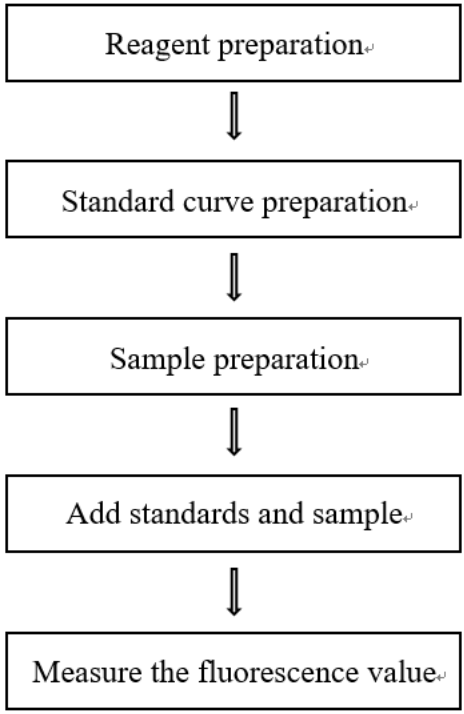
Assay summary

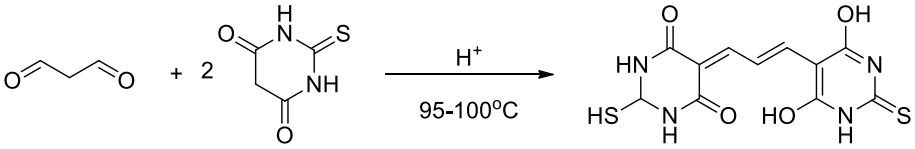


Intended use

This kit can be used to measure the malondialdehyde (MDA) content in serum, plasma, animal tissue, and cell samples.

Detection principle

MDA in the catabolite of lipid peroxide can react with thiobarbituric acid (TBA) and produce red compound, which is proportional to the fluorescence intensity at the excitation wavelength of 520 nm and emission wavelength of 550 nm.



Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Clarificant	6 mL ×1 vial	12 mL × 1 vial	2-8 ℃, 12 months
Reagent 2	Acid Reagent	2 mL ×1 vial	4 mL × 1 vial	2-8 ℃, 12 months
Reagent 3	TBA Reagent	Powder ×1 vial	Powder × 1 vial	2-8 ℃, 12 months shading light
Reagent 4	20 μmol/L Standard	5 mL ×1 vial	5 mL × 1 vial	2-8 ℃, 12 months
Reagent 5	CAMT Lysis Buffer	20 mL ×1 vial	40 mL × 1 vial	2-8 ℃, 12 months
	Black 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Micropipettor, Water bath, Vortex mixer, Centrifuge, Fluorescence microplate reader (Ex/Em=520 nm/550 nm)

Reagents:

Acetic acid, Normal saline (0.9% NaCl) or PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use. Frozen storage(2-8 ℃)may cause the clarificant to freeze. To re-dissolve place in a water bath (37 ℃) and until the clarificant looks clear.

② The preparation of acid application solution:
Before testing, please prepare sufficient acid application solution according to the test wells. For example, prepare 176 μL of acid application solution (mix well 6 μL of acid reagent and 170 μL of double distilled water). The acid application solution should be prepared on spot.

③ The preparation of TBA application solution:
Dissolve one vial of TBA reagent with 10 mL of clarificant, then add 10 mL of acetic acid, mix fully and cool to room temperature. Store at 2-8 ℃ for 1 month protected from light. (Acetic acid, analytical reagent, acetic acid concentration ≥99.5%. This reagent should be self-prepared.)

④ The preparation of chromogenic agent:
For each well, prepare 500 μL of chromogenic agent (375 μL of acid reagent application solution and 125 μL of TBA reagent application solution). The chromogenic agent should be prepared on spot and used up within 1 day.

⑤ The preparation of standard curve：

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

Dilute 20 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 4, 6, 8, 10 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**
20 μmol/L standard (μL)	0	25	50	100	200	300	400	500
Double distilled water (μL)	1000	975	950	900	800	700	600	500

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 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).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 - 1×10^7 cells).

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

③ Lyse 1×10^6-10^7 cells with 0.3 mL CAMT lysis buffer. Place on the ice box and crack for 10 min, then preserve it on ice for detection.

④ 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	2-4
Human plasma	1
Rat serum	1
Rat plasma	1
Mouse serum	2-4
Mouse plasma	1
10% Mouse brain tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Rat kidney tissue homogenate	2-4
9.2×10^6 CHO cells	1

Note: The diluent of serum (plasma) or animal tissue is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4); The diluent of cells is CAMT lysis buffer. 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 95-100℃ 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 Water-bath temperature (95-100℃) and incubation time (40 min) should be stabilized.

③ After lysis of cell samples, the suspension is directly taken for detection without centrifugation.

④ It is recommended to do pre-tested to determine the appropriate amount of cells.

Operating steps

① Standard tube: Take 0.1 mL of standard solution with different concentrations to the 1.5 mL EP tubes.

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

② Add 0.1 mL of clarificant into each tube.

③ Add 0.5 mL of chromogenic agent into each tube.

④ Mix fully with a vortex mixer. Make a hole in the tube mouth. Incubate the tubes in 95-100 ℃ water bath for 40 min.

⑤ Cool the tubes to room temperature with running water, centrifuge the tubes at 10000×g for 10 min.

⑥ Take 0.25 mL the supernatant of each tube to the microplate with a micropipette (the precipitation cannot be added to the microplate).

⑦ Measure the fluorescence intensity at the excitation wavelength of 520 nm and the emission wavelength of 550 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence 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:

$$\text{MDA (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue and cell samples:

$$\text{MDA (μmol/gprot)} = (\Delta F - b) \div a \times f \div C_{pr}$$

[Note]

ΔF: The absolute fluorescence value of sample ($F_{Sample} - F_{Blank}$).

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, gprot/L.

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)	0.80	4.50	7.30
%CV	4.3	3.3	2.3

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)	0.80	4.50	7.30
%CV	4.5	7.0	5.6

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 97%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	0.7	3.5	7.5
Observed Conc. (μmol/L)	0.7	3.5	7.3
Recovery rate (%)	95	99	97

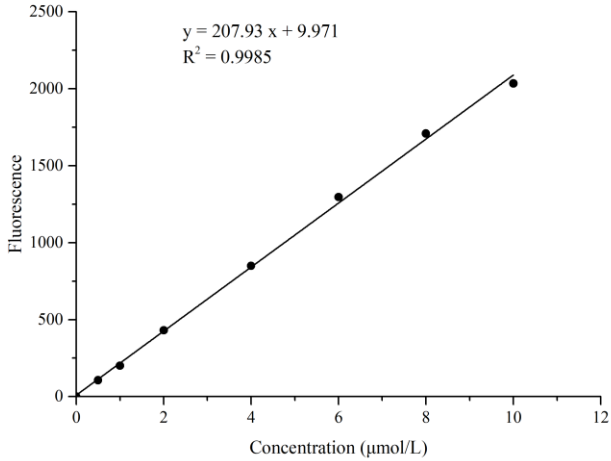
Sensitivity

The analytical sensitivity of the assay is 0.04 μmol/L. 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 fluorescence 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	0.5	1	2	4	6	8	10
Fluorescence value	57	164	262	486	893	1339	1780	2105
	62	168	258	495	924	1373	1757	2083
Average fluorescence value	59	166	260	490	909	1356	1768	2094
Absoluted fluorescence value	0	107	201	431	850	1297	1709	2035



Appendix II Example Analysis

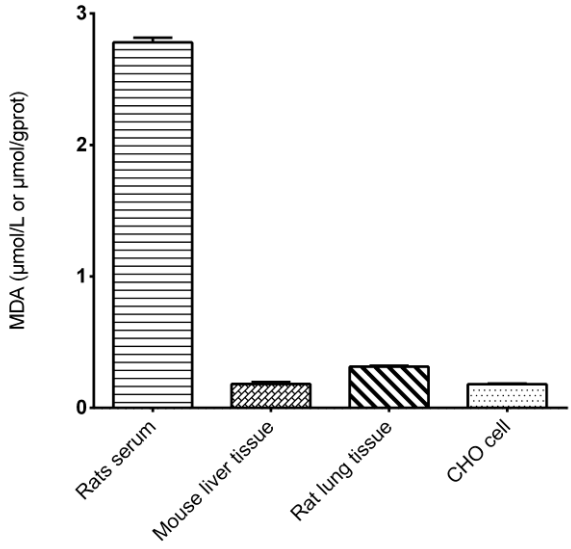
Example analysis:

Take 0.1 mL of 10% mouse liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 207.93 x + 9.971, the average fluorescence value of the sample is 545.27, the average fluorescence value of the blank is 59.14, the concentration of protein in sample is 12.59 gprot/L, and the calculation result is:

MDA (μmol/gprot) = (545.27 – 59.14 – 9.971) ÷ 207.93 ÷ 12.59 = 0.182 μmol/gprot

Detect rat serum (dilute for 3 times), 10% mouse liver tissue homogenate (the concentration of protein is 12.59 gprot/L), 10% rat lung tissue homogenate (the concentration of protein is 7.83 gprot/L) and 9.2×10^6 CHO cells (the concentration of protein is 7.24 gprot/L) 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.