Technical Manual

Malondialdehyde (MDA) Colorimetric Assay Kit (TBA Method)

- Catalogue Code: MAES0037
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

2.92-40 μmol/L

Sensitivity:

1.13 μmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

The body produce oxygen free radicals through the enzyme system and non-enzyme system, which can attack unsaturated fatty acid on biofilm and lead to lipid peroxidation and form lipid peroxide, such as aldehyde group (MDA), keto-, hydroxyl, carbonyl, etc. Oxygen free radicals cause cell damage not only by peroxidation of polyunsaturated fatty acids in biofilm, but also by decomposition products of lipid hydroperoxide. Detection of the MDA content can reflect the level of lipid peroxidation in cells and reflect level of cellular damage indirectly.

3. Intended Use

This kit can be used to measure the MDA content in serum, plasma, tissue and other samples.

4. 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.

5. Kit components & storage

Item	Specification	Storage
Clarificant	3 mL × 1 vial	2-8°C, 6 months
Acid Reagent	1.8 mL × 2 vials	2-8°C,6 months
Chromogenic Agent	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard (50 μmol/L)	5 mL × 1 vial	2-8°C,6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (530-540 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)
- Acetic acid
- Absolute ethanol

6. Assay Notes:

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.

4. In general, the serum (plasma) samples are no hemolysis or lipidemia, control tube can be remove, just need to establish blank (the concentration of standard is 0 μmol/L) tube.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

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

3. Acid application solution: Dilute acid reagent with double-distilled water at a ratio of 1.2: 34 and mix fully.

4. Chromogenic agent application solution: Dissolve the lyophilized of chromogenic agent with 14 mL of double-distilled water (90~100°C) fully, then add 14 mL of glacial acetic acid, mix fully and cool to room temperature. The prepared solution can be store at 4°C with avoid direct sunlight for 1 month. (Glacial acetic acid, analytical reagent, acetic acid concentration ≥99.5%. This reagent should be self-prepared.)

5. Preparation of 50% acetic acid
   Add 8mL of glacial acetic acid into 8mL of double distilled water slowlly and mix fully. Stand at room temperature for detection (Note: Glacial acetic acid with high concentr ations , please add slowly during the dilution process)

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (2.92-40 μmol/L).

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);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 532 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute standard (50 μmol/L) with absolute ethyl alcohol to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 μmol/L.

The measurement of samples

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

2. Add 0.02 mL of clarificant into each tube of Step 1.

3. Add 0.6 mL of acid application solution into each tube of Step 2.

4. Add 0.2 mL of chromogenic agent 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 100°C 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 value at 532 nm with microplate reader.

Operation Table

	Standard tube	Sample tube	Control tube
Standards with different concentrations (mL)	0.02		
Sample (mL)		0.02	0.02
Clarificant (mL)	0.02	0.02	0.02
Acid application solution (mL)	0.6	0.6	0.6
Chromogenic agent application solution (mL)	0.2	0.2	
50% Acetic acid (mL)			0.2
Mix fully, fasten the tube mouth with fresh-keeping film, mix fully, and make a small hole in the film. Then incubate the tubes at 100℃ for 40 min. Cool the tubes to room temperature with running water, centrifuge the tubes at 9569 g for 10 min. Take 0.25 mL the supernatant of each tube to the microplate with a micropipette. Measure the OD value at 532 nm with microplate reader.			

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) sample:

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

2. Tissue and cells sample:

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

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve.
ΔA: $OD_{Sample} - OD_{Blank}$/ $OD_{Control}$
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

12. Performance Characteristics

Detection Range	2.92-400 μmol/L
Sensitivity	1.13 μmol/L
Average recovery rate (%)	97.8
Average inter-assay CV (%)	7.2
Average intra-assay CV (%)	4.1

Analysis

Take 0.02 mL of 10% rat liver tissue homogenate, 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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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