Malondialdehyde (MDA) Colorimetric Assay Kit (TBA Method)

Catalog Number EEA015 (96 tests)

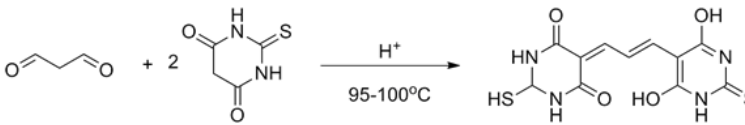
Rev 2.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can be used to measure the MDA content in serum, plasma and tissue samples. The body produces oxygen free radicals through the enzyme system and non-enzyme system, which can attack unsaturated fatty acids on biofilm and lead to lipid peroxidation to form lipid peroxides, 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 via decomposition products of lipid hydroperoxide. Detection of MDA content can reflect the level of lipid peroxidation in cells and indirectly reflect the level of cellular damage.

MDA is the catabolite of lipid peroxide, reacts with thiobarbituric acid (TBA) to produce a red compound, which has a maximum absorption peak at 532 nm.



Contents and storage

Kit and components are shipped at 2-8 ℃. An unopened kit can be stored at 2-8 ℃ for 12 months.

Components	Quantity (96 tests)
Clarificant	3 mL
Acid Reagent	4 mL
Chromogenic Agent	Powder
50 μmol/L Standard	5 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Glacial acetic acid, analytical reagent, acetic acid concentration ≥99.5%
- Absolute ethanol
- Microtiter plate reader with software capable of measurement at or near 530-540 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37 ℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8 ℃ to remove blood cells.
- 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 ℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4 ℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227).

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80 ℃ for 1 month.

Prepare samples

It is recommended to take 2-3 samples with expected large difference to do a pre-experiment before formal experiment and 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).

Clarificant

Clarificant will be frozen when stored at 2-8 ℃ for longer periods, thaw reagent in 37 ℃ water-bath until clear before use.

Preparation of acid reagent application solution

Dilute acid reagent with distilled or deionized water at a ratio of 1.2: 34 and mix fully.

Preparation of chromogenic agent

Dissolve the powder with 14 mL of distilled or deionized (90-100 ℃) fully, then add 14 mL of glacial acetic acid, mix fully and cool to room temperature. The prepared solution can be stored at 4 ℃ away from light for 1 month.

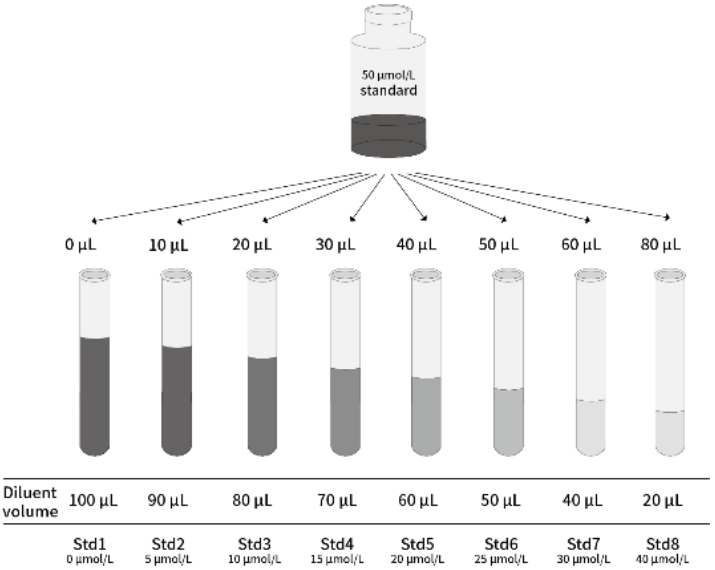
Preparation of 50% acetic acid

Add 8 mL of glacial acetic acid into 8 mL of distilled or deionized water slowly and mix fully. Keep at room temperature for detection (Note: Glacial acetic acid with high concentrations, please add slowly during the dilution process).

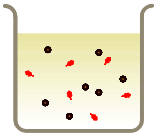
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Dilute 50 μmol/L pyruvic acid standard solution with distilled or deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 μmol/L.



Assay Procedure

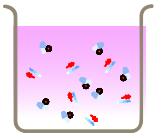


1. Add sample, standard and control

a. Standard tube: take 0.02 mL of the standard solution with different concentrations into numbered 1.5 mL tubes.

b. Sample tube: take 0.02 mL of sample into numbered 1.5 mL tubes.

c. Control tube: take 0.02 mL of sample into numbered 1.5 mL tubes.



2. Add substrate

a. Add 0.02 mL of the clarificant into each tube.

b. Add 0.6 mL of the acid reagent application solution into each tube.

c. Add 0.2 mL of the chromogenic agent application solution into standard tubes and sample tubes. Add 0.2 mL of 50% acetic acid to the control tubes.

d. Fasten the tube mouths with wrapping film, mix fully, and make a small hole in the film. Then incubate the tubes at 100 ℃ for 40 min.

e. Cool the tubes to room temperature with running water, then centrifuge the tubes at 9569 g for 10 min.

f. Add 0.25 mL of the supernatant from each tube to the microplate with a micropipette (careful to not add the precipitate to the wells).

g. Measure the OD values of each well with a microplate reader at 532 nm.

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 (Std1) 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).

Serum (plasma) and other liquid sample:

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

Tissue sample:

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

[Note]

y: The absolute OD value of standard;

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔA: Absolute OD, $OD_{Sample} - OD_{Blank}$. (Note:The ΔA value of hemolysis or lipidemia serum (plasma) sample:OD_{sample}-$OD_{control}$).

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (gprot/L)

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

Take 0.02 mL of 10% rat liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

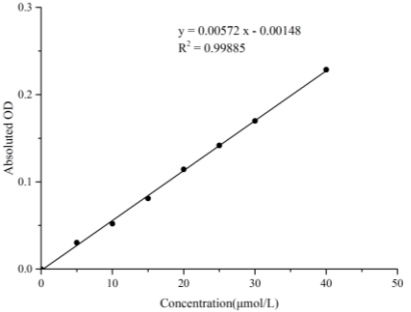
standard curve: y = 0.0057 x – 0.0015, the average OD value of the sample is 0.075, the average OD value of the blank is 0.041, the concentration of protein in sample is 12.89 gprot/L and the calculation result is:

$$\text{MDA (μmol/gprot)} = (0.075 - 0.041 + 0.0015) \div 0.0057 \div 12.89 = 0.48 \text{ μmol/gprot}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0-40 µmol/L standard.

Concentration (µmol/L)	OD at 532 nm	Standard Curve
40	0.269	
30	0.21	
25	0.182	
20	0.155	
15	0.122	
10	0.093	
5	0.071	
0	0.041	

- Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	4.60	25.30	32.50
%CV	4.4	4.0	3.9

CV = Coefficient of Variation

- 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

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum, and plasma samples

Sample Type	Range (**μmol/L**)	Average (**μmol/L**)
Human serum	9.0-13.2	11.66
Rat plasma	3.1-5.4	4.02

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

- Recommended 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.

- Sensitivity

The analytical sensitivity of the assay is 1.13 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.

Limited product warranty

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