TECHNICAL MANUAL

Malondialdehyde (MDA) Colorimetric Assay Kit (TBA)

- **SKU CODE:** MAES0340
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

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

2. Detection principle

MDA in the catabolite of lipid peroxide can react with thiobarbituric acid (TBA) and produce a red compound, which has a maximum absorption peak at 532 nm.

3. Kit components & storage

Item	Component	Size (100 assays)	Storage
Reagent 1	Clarificant	24 mL x 1 vial	2-8 °C, 12 months
Reagent 2	Acid Reagent	12 mL x 1 vial	2-8 °C, 12 months
Reagent 3	Chromogenic Agent	Powder x 2 vials	2-8 °C, 12 months, shading light
Reagent 4	10 nmol/mL Standard	5 mL x 1 vial	2-8 °C, 12 months

4. 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

5. Reagent preparation

1. Clarificant may solidify during frozen storage (2-8°C). To re-dissolve, place in a water bath (37 °C) until the clarificant looks clear. Equilibrate other reagents to room temperature before use.
2. Preparation of acid application solution: For each tube, prepare 3.0 mL of acid application solution by thoroughly mixing 102.3 µL of acid reagent with 2897.7 µL of double distilled water.
3. Preparation of chromogenic application solution: Dissolve the powder completely with 30 mL of double distilled water (90-100 °C), then add 30 mL of glacial acetic acid, mix thoroughly and cool to room temperature. Store at 4 °C for 1 month protected from light.

6. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C 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 °C.
4. Centrifuge at 10000xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

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 perform a pre-test to confirm the dilution factor.

7. 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 °C) 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.

8. 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 thoroughly and fasten the mouth of the tube with plastic film, prick a small hole with a needle. Then incubate the tubes at 95-100 °C for 40 min.
6. Cool the tubes to room temperature with running water, centrifuge the tubes at 3100 xg for 10 min.
7. Take 3 mL of the supernatant from 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).

9. Calculation

The sample:

1. Serum (plasma) sample:

MDA content (nmol/mL) = (ΔA_1 / ΔA_2) × c × f

2. Tissue sample:

MDA content (nmol/mgprot) = (ΔA_1 / ΔA_2) × c × f ÷ 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

10. Appendix I Performance Characteristics

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

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 (nmol/mL)	1.20	35.60	102.50
%CV	7.8	8.2	8.0

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with 6 parallel measurements for each concentration to obtain an 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.

11. Appendix II Example Analysis

Example analysis:

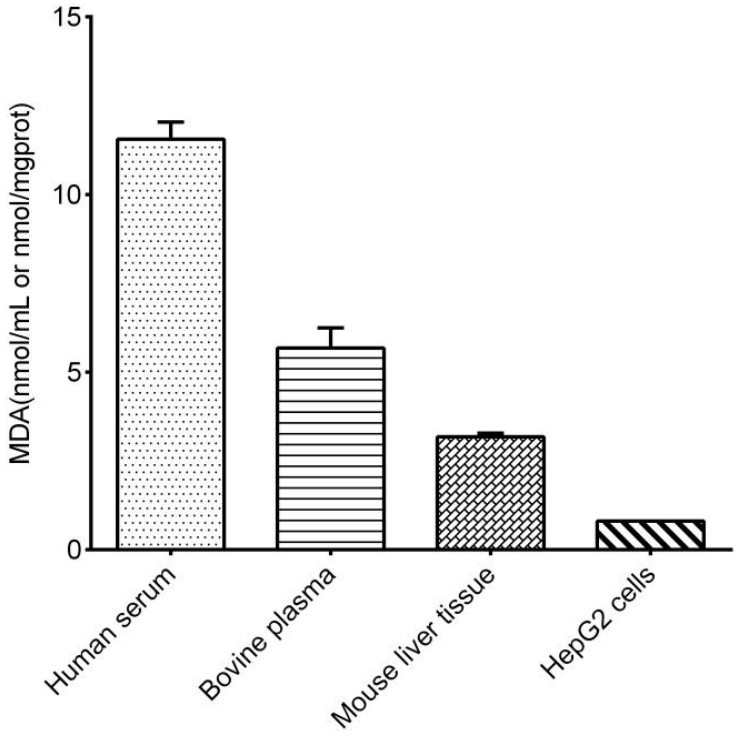
Take 0.1 mL of human serum and carry out the assay according to the operation table.

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:

MDA content (nmol/mL) = (0.069 - 0.002) / (0.060 - 0.002) × 10 = 11.55 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 results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.