TECHNICAL MANUAL

Lipid Peroxidation (MDA) Colorimetric Assay Kit (TBA)

- **SKU CODE:** MAES0037
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples to tubes
- Add reagents and incubate
- Measure optical density

2. Intended use

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

3. Detection principle

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

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3 (500 Assays)	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	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. 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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. Clarificant will freeze when stored at 2-8°C for extended periods. Warm it in a 37°C water bath until clear before use.
3. **Preparation of acid application solution:** Before testing, prepare sufficient acid application solution according to the number of test tubes. For example, to prepare 616 µL of acid application solution, thoroughly mix 21 µL of acid reagent with 595 µL of distilled or deionized water.
4. **Preparation of chromogenic application solution:** Dissolve the chromogenic agent with 14 mL of hot distilled or deionized water (90-100°C), then add 14 mL of glacial acetic acid and mix thoroughly. Cool to room temperature. The prepared solution can be stored at 4°C protected from light 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:** For each tube, prepare 0.2 mL of 50% acetic acid by mixing 100 µL of glacial acetic acid with 100 µL of distilled or deionized water. Add glacial acetic acid slowly during dilution.
6. **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 create a series of concentrations. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 µmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one 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 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of the supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

- 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. During incubation in the 100°C water bath, EP tubes should not be closed directly. It is recommended to fasten the tube mouth with plastic wrap and make a small hole in the film.
2. The water bath temperature and incubation time should be stable (95-100°C, 40 min).

3. The supernatant for colorimetric measurement should not contain sediment, as this will affect OD values. It is recommended to use a pipette to collect the supernatant.

9. 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 to standard tubes and sample tubes. Add 0.2 mL of 50% acetic acid to control tubes.
5. Fasten the tube mouth with plastic wrap, mix thoroughly, 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, then centrifuge at 9,569×g for 10 min.
7. Transfer 0.25 mL of the supernatant from each tube to the microplate using a micropipette (do not transfer any precipitation to the microplate).
8. Measure the OD values of each well at 532 nm with a microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using the absolute OD value of standards and corresponding concentrations 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

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

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

(Note: The ΔA value for hemolyzed or lipemic serum (plasma) samples: $OD_{Sample} - OD_{Control}$)

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

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.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	4.60	25.30	32.50
%CV	8.0	6.9	6.7

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 97.8%.

Recovery

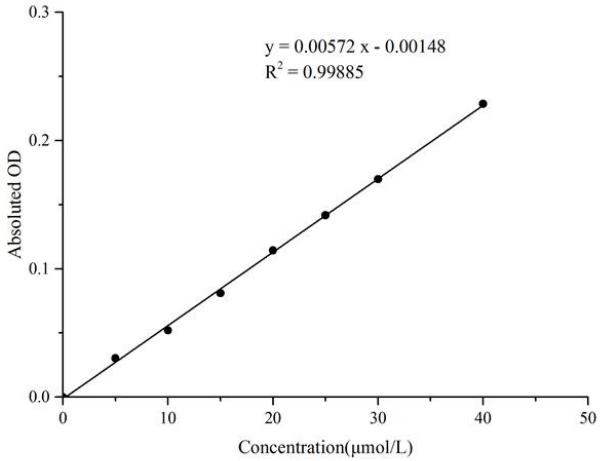
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	12	18	29
Observed Conc. (µmol/L)	11.6	17.8	28.3
Recovery rate (%)	96.9	99	97.5

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.

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), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μmol/L)	Average OD	Absolute OD
0	0.041	0
5	0.071	0.030
10	0.093	0.052
15	0.122	0.081
20	0.155	0.114
25	0.182	0.142
30	0.210	0.170
40	0.269	0.229

12. Appendix II Example Analysis

Example analysis:

Take 0.02 mL of 10% rat liver tissue homogenate and perform the assay according to the operating procedure. The results are as follows:

Standard curve: y = 0.0057x – 0.0015

Average OD value of the sample: 0.075

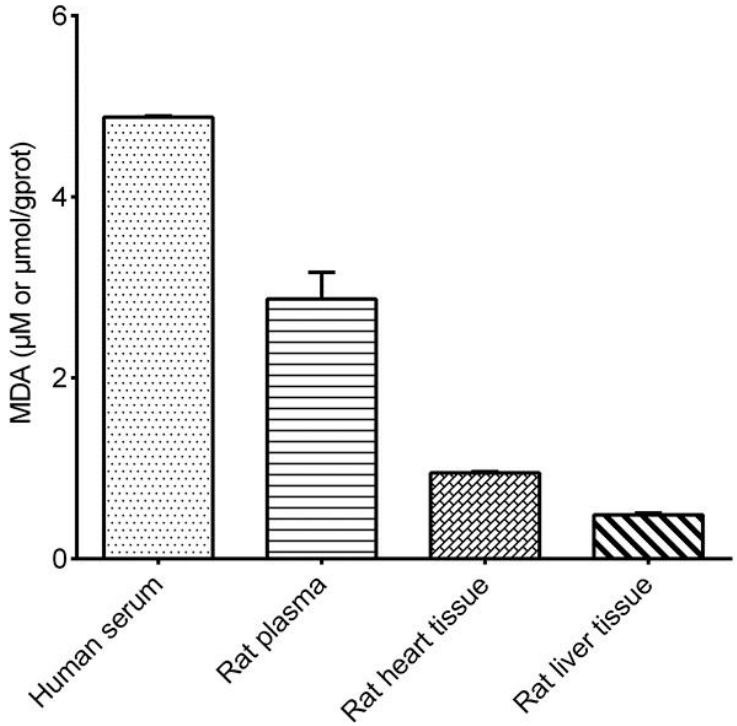
Average OD value of the blank: 0.041

Concentration of protein in sample: 12.89 gprot/L

Calculation result:

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 (protein concentration: 6.21 gprot/L), and 10% rat liver tissue homogenate (protein concentration: 12.89 gprot/L) 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 experiments. Follow the instructions strictly throughout the procedure.

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

4. If the concentration of the substance is not within 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. The 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!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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