TECHNICAL MANUAL

Malondialdehyde (MDA) Fluorometric Assay Kit

- **SKU CODE:** MAES0274
- **SIZE:** 48T/96T
- **DETECTION PRINCIPLE:** Colorimetric
- **RUO:** Research-Use-Only

Revised: 08/2025

Malondialdehyde (MDA) Fluorometric Assay Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Measuring instrument:

Fluorescence microplate reader (Ex/Em=520 nm/550 nm)

Detection range:

0.04-10 μmol/L

Sample type:

Serum, plasma, animal tissue, and cell samples.

2. Storage & Expiry

This product should be stored at 2-8 ^{O}C in dark conditions for up to 12 months. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

Malondialdehyde (MDA) is a highly reactive aldehyde generated as a major end-product of lipid peroxidation, a process in which reactive oxygen species (ROS) attack polyunsaturated fatty acids in cell membranes. Because of its stability and abundance, MDA is widely recognized as a reliable biomarker of oxidative stress and cellular damage. Elevated MDA levels are associated with various pathological conditions, including cardiovascular disease, neurodegeneration, diabetes, cancer, and aging-related disorders.

In this assay, MDA reacts with thiobarbituric acid (TBA) to form a stable red-colored adduct. The intensity of this adduct is directly proportional to the amount of MDA present in the sample. Quantification is performed fluorometrically, with excitation at 520 nm and emission at 550 nm, providing a sensitive and specific means of assessing lipid peroxidation and oxidative stress levels in biological samples.

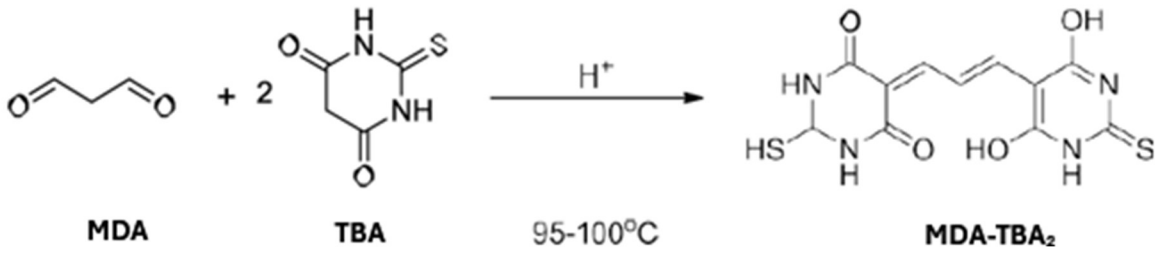


Figure 1. Chemical reaction scheme for the detection of malondialdehyde (MDA) using the MDA Fluorometric Assay Kit .

4. Kit Contents

No	Component Name	Size (48T)	Size (96T)	Storage
1	Clarificant	6 mL x 1 vial	12 mL x 1 vial	2-8 °C, 12 months
2	Acid Reagent	2 mL x 1 vial	4 m L x 1 vial	
3	TBA Reagent	Powder x 1 vial	Powder x 1 vial	2-8 °C, 12 months (protect from light)
4	20 µmol/L Standard	5 mL x 1 vial	5 mL x 1 vial	2-8 °C, 12 months
5	CAMT Lysis Buffer	20 mL x 1 vial	40 m L x 1 vial	
6	Black Microplate	96 wells		
7	Plate Sealer	2 pieces		

Note: *All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.*

Additional materials required:

1. 37°C incubator.
2. Fluorescence microplate reader (Ex/Em=520 nm/550 nm).
3. Vortex
4. Centrifuge
5. Precision pipettes and disposable pipette tips. Distilled water.
6. Disposable tubes for sample dilution.
7. Absorbent paper.

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. Reagent Preparation

1. **Equilibrate Reagents**: Bring all reagents to room temperature before use. Storage at 2–8 °C may cause the clarificant to precipitate. If this occurs, re-dissolve by placing the reagent in a 37 °C water bath until the solution becomes completely clear.
2. **Preparation of Acid Application Solution**: Immediately before testing, prepare sufficient acid application solution according to the number of test wells required. For example, to prepare 176 µL of acid application solution, mix 6 µL of acid reagent with 170 µL of double-distilled water. The acid application solution must be freshly prepared and used immediately.
3. **Preparation of TBA Application Solution**: Dissolve one vial of TBA reagent in 10 mL of double-distilled water pre-heated to 90–100 °C, and mix thoroughly until fully dissolved. Add 10 mL of glacial acetic acid (analytical grade, ≥ 99.5%) and mix well. Allow the solution to cool to room temperature. Store the prepared TBA solution at 2–8 °C, protected from light, for up to 1 month. ***Note:*** *Acetic acid solution must be self-prepared*.
4. **Preparation of Chromogenic Agent:** For each well, prepare 500 µL of chromogenic agent by mixing 375 µL of acid application solution with 125 µL of TBA application solution. The chromogenic agent must be freshly prepared and used within the same day.
5. **Preparation of Standard Curve**: Always prepare a fresh standard curve for each assay. Discard all working dilutions after use. Prepare serial dilutions of the 20 µmol/L standard solution using double-distilled water. The recommended concentration gradient is: 0, 0.5, 1, 2, 4, 6, 8, and 10 µmol/L.

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

7. Sample preparation

A. Sample Preparation

Serum or Plasma Sample

Serum and plasma can be tested directly. If immediate testing is not possible, samples may be stored at –80 °C for up to one month.

Tissue Sample:

1. Harvest the required amount of tissue for each assay (recommended starting amount: 20 mg).
2. Wash the tissue thoroughly with cold PBS (0.01 M, pH 7.4).
3. Homogenize 20 mg of tissue in 180 µL of PBS (0.01 M, pH 7.4) using a Dounce homogenizer at 4 °C.
4. Centrifuge the homogenate at 10,000 × g for 10 minutes to remove insoluble material. Collect the supernatant and keep it on ice for subsequent detection.
5. Determine the protein concentration of the collected supernatant prior to analysis.

Cell (adherent or suspension) Samples:

1. Harvest the required number of cells for each assay (recommended: 1 × 10^6 to 1 × 10^7 cells).
2. Wash the cells with PBS (0.01 M, pH 7.4).
3. Lyse 1 × 10^6 to 1 × 10^7 cells in 0.3 mL CAMT lysis buffer. Place the lysate on ice for 10 minutes to ensure complete disruption, and keep on ice until detection.
4. Determine the protein concentration of the supernatant prior to analysis.

B. Dilution

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.

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% Mouse 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 recommended diluent is the provided buffer solution. For sample types not specified in the protocol, it is advised to perform a preliminary test to determine the appropriate dilution factor or contact our Tech Support Team at techsupport@assaygenie.com.*

8. Assay Procedure

Before starting:

1. **Sample and Standard Preparation:**
    a. For the standard tubes: Pipette 0.1 mL of each standard solution (different concentrations) into separate 1.5 mL Eppendorf (EP) tubes.
    b. For the sample tubes: Pipette 0.1 mL of each sample into separate 1.5 mL EP tubes.
2. **Reagent Addition:** Add 0.1 mL of clarificant to each tube, followed by 0.5 mL of chromogenic agent.
3. **Incubation:** Mix thoroughly using a vortex mixer. Pierce the cap of each tube to prevent pressure buildup, then incubate the tubes in a 95–100 °C water bath for 40 minutes.
4. **Cooling and Centrifugation:** Cool the tubes to room temperature under running water. Centrifuge at 10,000 × g for 10 minutes.
5. **Supernatant Collection:** Transfer 0.25 mL of the supernatant from each tube to a microplate well using a micropipette. Avoid transferring any precipitate.
6. **Fluorescence Measurement:** Measure the fluorescence intensity at an excitation wavelength of 520 nm and an emission wavelength of 550 nm.

9. Data Analysis

Calculate the average OD value for each standard concentration from the duplicate wells. Subtract the mean OD value of the blank (Standard **#1**, 0.0 mmol/L) from all standard readings to obtain the corrected (absolute) OD values.

Plot the standard curve by placing the corrected OD values on the y-axis and the corresponding standard concentrations on the x-axis. Generate the linear regression equation (y = ax + b) using graphing software or Microsoft Excel.

The sample:

1. Serum or Plasma Samples

MDA (μmol/L) = (ΔF - b) ÷ a × f

2. Tissue and cell samples:

MDA (μmol/gprot) = (ΔF - b) ÷ a × f ÷ Cpr

Note:

ΔF: The absolute fluorescence value of sample (FSample – FBlank). f: Dilution factor of sample before tested.

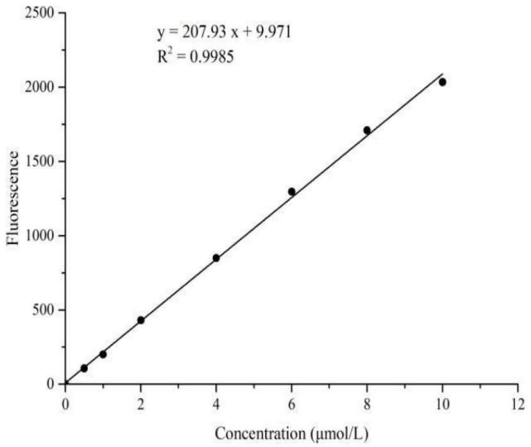
Cpr: Concentration of protein in sample, gprot/L.

10. Typical Data

Standard Curve

The OD values of the standard curve may vary depending on specific assay conditions, such as operator technique, pipetting accuracy, and temperature fluctuations. Therefore, the standard curve and data provided below are for reference only and should not be used for direct result calculation. Always generate a fresh standard curve for each assay run.

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
Normalized fluorescence value	0	107	201	431	850	1297	1709	2035



Sensitivity

The analytical sensitivity of the assay is 0.04 µmol/L. This value was determined by measuring the zero standard (blank) in 20 independent replicates, calculating the mean OD and adding two standard deviations. The corresponding concentration was then derived from the standard curve.

Recovery

Three sample concentrations (high, medium, and low) were tested in parallel, with six replicates per concentration. The average recovery rate across all concentrations was determined to be 97%.

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

Intra-assay Precision

Three human serum samples were assayed in replicates of [VALUE] 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

11. Example Analysis

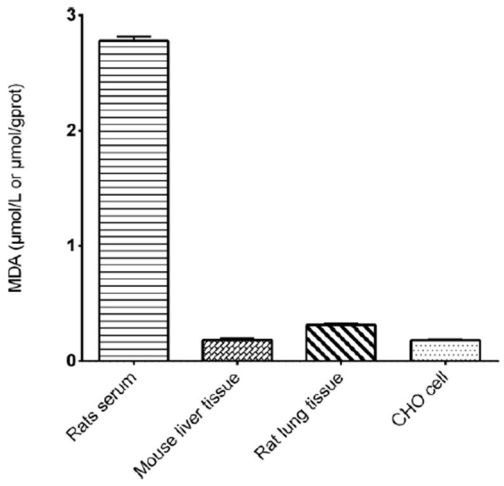
Mouse Liver Tissue (10% Homogenate) Detection Results:

- **Sample volume:** 0.1 mL
- **Standard curve:** *y = 207.93x + 9.971*
- **Average fluorescence value (sample):** 545.27
- **Average fluorescence value (blank):** 59.14
- **Protein concentration:** 12.59 g/L
- **Calculation:**

$$\text{MDA (μmol/g protein)} = \frac{(545.27–59.14–9.971)}{207.93 \times 12.59} = 0.182\,\mu\text{mol/g protein}$$

Additional Sample Types:

- **Rat Serum**: Diluted 3-fold before assay.
- **Mouse Liver Tissue (10% homogenate)**: protein concentration = 12.59 g/L.
- **Rat Lung Tissue (10% homogenate)**: protein concentration = 7.83 g/L.
- **CHO Cells (9.2 × 10^6 cells)**: protein concentration = 7.24 g/L.



Notes:

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.