Malondialdehyde Assay Kit

Catalog No.: abx092294

Size: 96 tests

Detection Range: 0.04 µmol/ml – 10 µmol/ml

Sensitivity: 0.04 µmol/ml

Storage: Store all components between 2 – 8°C. Store the TBA Reagent in the dark.

Application: For detection and quantification of Malondialdehyde concentration in serum, plasma, cell lysates and tissue homogenates.

Introduction

Abbexa's Malondialdehyde Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Malondialdehyde concentration. Malondialdehyde reacts with thiobarbituric acid (TBA) to produce a red-colored compound. This compound has a characteristic excitation/emission wavelength of 532/553 nm. The intensity of the fluorescence emitted is directly proportional to the Malondialdehyde concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Clarifying Reagent: 12 ml
3. Acidic Reagent: 4 ml
4. TBA Reagent: 1 vial
5. Standard (20 µmol/ml): 5 ml
6. CAMT Lysis Buffer: 40 ml
7. Plate sealers: 2

Materials required but not provided

1. Fluorescence microplate reader (Ex/Em=520 nm/550 nm)
2. Double distilled water
3. PBS (0.01 M, pH 7.4)
4. Normal Saline (0.9% NaCl)
5. Acetic acid
6. Pipette and pipette tips
7. Microcentrifuge tubes
8. Centrifuge
9. Vortex mixer
10. Mechanical homogenizer
11. Incubator
12. Timer
13. Water bath

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation method is intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and plasma**: Serum and plasma samples can be tested directly. Avoid hemolytic samples.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Add the tissue to 180 µl of PBS (0.01 M, pH 7.4) and homogenize manually using a mechanical homogenizer at 4°C. Collect the supernatant and store on ice for detection.
- **Cell Lysates:** Harvest 1 × 10^6 - 1 × 10^7 cells and wash them with PBS (0.01M, pH 7.4). Add cells to 300 µl of CAMT Lysis Buffer and keep on ice for at least 10 minutes. Keep sample on ice for immediate detection.

Note: To calculate Malondialdehyde concentration in tissue homogenates and cell lysates, using the formulae in section C. Calculation of Results, the total protein concentration of the supernatant must be determined separately (abx097193).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute serum, plasma and tissue samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Dilute cell lysates in CAMT lysis buffer. The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Recommended 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% Rat 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:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

2. Reagents

- **Clarifying Reagent:** Preheat to 37°C in a water bath, the liquid should be transparent before use.
- **Acidic Working Solution:** For each well tested, prepare 176 µl of Acidic Working Solution. Add 6 µl of Acidic Reagent to 170 µl of double-distilled water and mix thoroughly. Prepare for immediate use.
- **TBA Working Solution:** Add 1 vial of TBA Reagent in 10 ml of pre-heated Clarifying Reagent and 10 ml of acetic acid and mix thoroughly. Allow to cool to room temperature before use. Unused TBA Working Solution can be stored between 2 – 8 °C for up to 1 month in the dark.
- **Chromogenic Reagent:** For each well to be assayed, prepare 500 µl of Chromogenic Reagent. Add 375 µl of Acidic Working Solution to 125 µl TBA Working Solution. Use within 24 hours..
- **Standards:** Prepare standard dilutions as summarized in the following table:

Standard concentration (µmol/ml)	**0**	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**
20 µmol/ml Standard (µl)	0	25	50	100	200	300	400	500
Double-distilled water (µl)	1000	975	950	900	800	700	600	500

For the blank, or 0 nmol/ml standard, use Double distilled water. The volume of each standard will be 1000 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

Pre-heat the water bath to 95 - 100 °C. Ensure a stable temperature is reached prior to use.

1. Label sterile microcentrifuge tubes for samples and standards. *It is recommended to test each tube in duplicate.*
2. Add 100 µl of sample to the sample tubes.
3. Add 100 µl of standard dilutions to the standard tubes.
4. Add 100 µl of Clarifying Reagent to all tubes.
5. Add 500 µl of Chromogenic Reagent to all tubes and mix thoroughly using a vortex mixer.
6. Seal the tubes with adhesive film and pierce a small hole in the film.
7. Incubate all tubes at 95 - 100°C in a water bath for 40 minutes.
8. Cool the tubes to room temperature with running water, then centrifuge at 1,000 × g for 10 minutes.
9. Assign and record microplate well locations for each sample and standard dilution.
10. Carefully pipette 250 µl of supernatant from each tube, without disturbing the sediment, to the microplate.
11. Measure the fluorescence intensity at the excitation wavelength of 520 nm and the emission wavelength of 550

nm.

C. Calculation of Results

Plot the standard curve, using the fluorescence of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Malondialdehyde in each sample well can be derived with the following formulae:

1. **Serum and plasma samples:**

$$\text{Malondialdehyde concentration (µmol/L)} = \frac{\Delta F - b}{a} \times \text{f}$$

2. **Tissue homogenate and cell lysate samples:**

$$\text{Malondialdehyde concentration (µmol/g protein)} = \frac{\Delta F - \text{b}}{\text{a} \times \text{C}_{\text{Protein}}} \times \text{f}$$

Where:

ΔF	Absolute fluorescence value of sample ($F_{Sample} - F_{Blank}$)
a	Gradient of the standard curve ($y = \mathbf{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \mathbf{b}$)
f	Sample dilution factor
$C_{Protein}$	Concentration of protein in sample (g protein/ L)