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Instructions for Use

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Malondialdehyde (MDA) Assay Kit

Catalog No.: abx294021

Size: 100 tests

Detection Range: 0.38 nmol/ml – 133.33 nmol/ml

Sensitivity: 0.38 nmol/ml

Storage: Store all components at 4°C. Store the Chromogenic Reagent in the dark.

Application: For detection and quantification of Malondialdehyde content in serum, plasma, and tissue homogenates.

Principle of the Assay

Abbexa's Malondialdehyde (MDA) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Malondialdehyde content. In the presence of thiobarbituric acid, Malondialdehyde reacts to produce a red compound, with an absorbance maximum at 532 nm. The intensity of the color is proportional to the Malondialdehyde content, which can then be calculated.

Kit components

1. Clarifying Reagent: 24 ml
2. Acidic Reagent: 12 ml
3. Chromogenic Reagent: 2 vials
4. Standard (10 nmol/ml): 5 ml

Materials required but not provided

1. Spectrophotometer (532 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Glacial acetic acid
6. 50% acetic acid
7. Absolute (anhydrous) ethanol
8. Pipette and pipette tips
9. Glass test tubes
10. Centrifuge
11. Vortex mixer
12. Incubator

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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 methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh out at least 20 mg of tissue. Wash the tissue thoroughly in ice-cold PBS (0.01 M, pH 7.4). Add the tissue section into ice-cold PBS (0.01 M, pH 7.4) in a ratio of 1:9 weight (mg) to volume (µl) (i.e. for 20 mg of tissue, add into 180 µl ice-cold PBS). Homogenize manually, using a mechanical homogenizer or by ultrasonication, at 4°C. Centrifuge the resulting homogenate at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant, and keep on ice for detection. Detect immediately.

  Note: To calculate Malondialdehyde content in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum and plasma	1
Rat serum and plasma	1
Mouse serum and plasma	1
1% *Daucus carota* tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat kidney tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

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- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Working Acidic Reagent**: Prepare according to the volume required in Section **B. Assay Procedure**. Each tube tested will require 3 ml Working Acidic Reagent. To prepare 3 ml Working Acidic Reagent, add 102.3 µl Acidic Reagent into 2897.7 µl double-distilled water. Mix fully.
- **Working Chromogenic Reagent**: Reconstitute the Chromogenic Reagent powder in 30 ml of double-distilled water heated to >90°C. Add 30 ml glacial acetic acid, mix fully, and allow the solution to cool to room temperature before use. The Working Chromogenic Reagent can be stored for up to 1 month at 4°C in the dark.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- The Clarifying Reagent may partially solidify at low temperatures. If any solids are present, warm the vial to 37°C in a water bath until the Clarifying Reagent is a completely transparent liquid.

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B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark glass test tubes for each sample, control, standard, and blank. *It is recommended to test each tube in duplicate. Each sample requires a corresponding control.*
2. Add 0.1 ml sample into the sample tubes.
3. Add 0.1 ml sample into the corresponding control tubes.
4. Add 0.1 ml standard (10 nmol/ml) into the standard tubes.
5. Add 0.1 ml absolute ethanol into the blank tubes.
6. Add 0.1 ml Clarifying Reagent to all tubes.
7. Add 3 ml Working Acidic Reagent to all tubes.
8. Add 1 ml Working Chromogenic Reagent to the sample, standard, and blank tubes.
9. Add 1 ml 50% acetic acid to the control tubes.
10. Mix all tubes fully, then seal with plastic film. With a needle, prick small holes in the film. Incubate the tubes at 95°C – 100°C for 40 minutes. *Ensure the temperature is stable throughout the incubation.*
11. Cool the tubes quickly to room temperature in running water, then centrifuge the tubes at 3100 × g for 10 minutes.
12. Carefully take 3 ml of supernatant, without disturbing the sediment, and transfer to a fresh cuvette. Zero the spectrophotometer with double-distilled water.
13. Measure the OD of each cuvette at 532 nm with a 1 cm optical path.

C. Calculation of Results

The concentration of Malondialdehyde in each sample cuvette can be derived with the following formulae:

1. Serum and Plasma samples:

$$\text{Malondialdehyde (nmol/ml)} = \text{F} \times \text{C}_{\text{Standard}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}}$$

2. Tissue samples:

$$\text{Malondialdehyde (nmol/mg protein)} = \text{F} \times \text{C}_{\text{Standard}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{C}_{\text{Protein}} \times (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})}$$

where:

$\text{OD}_{\text{Sample}}$	OD value of sample
$\text{OD}_{\text{Standard}}$	OD value of standard
$\text{OD}_{\text{Control}}$	OD value of control
OD_{Blank}	OD value of blank
$\text{C}_{\text{Standard}}$	Concentration of standard (10 nmol/ml)
$\text{C}_{\text{Protein}}$	Concentration of protein in sample (mg/ml)
F	The dilution factor of sample

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