(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Microscale Malondialdehyde (MDA) Assay Kit

Catalog No.: BC00192

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Microscale Malondialdehyde (MDA) Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, plasma, cell culture medium, cell, tissue
Assay Type	Quantitative
Detection Instrument	Spectrophotometer, Microplate reader (532 nm)

Product Introduction

The organism generates oxygen free radicals via enzymatic and non-enzymatic systems. These free radicals attack polyunsaturated fatty acids (PUFAs) in biological membranes and trigger lipid peroxidation, thereby producing lipid peroxides, such as aldehyde groups (malondialdehyde, MDA), ketone groups, hydroxyl groups, carbonyl groups, hydroperoxyl groups, endoperoxyl groups, as well as newly generated oxygen free radicals.

Lipid peroxidation not only converts reactive oxygen species into reactive chemical agents, namely non-radical lipid degradation products, but also amplifies the effects of reactive oxygen through chain or branched-chain reactions. Consequently, a single initial reactive oxygen species can induce the formation of numerous lipid decomposition products. Among these products, some are harmless, while others may cause cellular metabolic disorders, functional impairment, and even cell death.

Oxygen free radicals induce cellular damage not only by peroxidizing polyunsaturated fatty acids (PUFAs) in biological membranes but also through the degradation products of lipid hydroperoxides. Therefore, the determination of MDA content can generally reflect the level of in vivo lipid peroxidation and indirectly indicate the degree of cellular damage.

Principle

Malondialdehyde (MDA), a degradation product of lipid peroxides, can condense with thiobarbituric acid (TBA) to form a red product with a maximum absorption peak at 532 nm. Since the substrate is thiobarbituric acid (TBA), this detection method is defined as the TBA method.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	20 mL	Stored at room temperature.
Reagent 2	Liquid	12 mL	Dilute with distilled water at a volume ratio of 6:170 to prepare the working solution before use. (It can also be prepared in a single batch.) Store at 4°C.
Reagent 3	Powder	1 vial	Before use, add the powder into 62 mL of distilled water, stir and heat to 90°C -100°C. After complete dissolution, add 60 mL of glacial acetic acid to prepare the working solution. Store at 4°C away from light.
Reagent 4	10 nmol/mL tetraethoxypropane standard	5 mL	Store at 4°C.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Note: Reagent 1 may solidify under refrigeration. Thaw by water bath heating at 37°C before use.

Storage

The kit shall be stored at 2-8°C with a validity period of 12 months. The prepared reagents can be stored for at least 6 months. It features stable color development, and the absorbance remains unchanged within 24 hours after color reaction.

Preparation

Visible spectrophotometer or microplate reader, constant temperature water bath or boiling water bath capable of adjusting to approximately 95°C, centrifuge, absolute ethanol (analytical grade), glacial acetic acid (concentration ≥ 99.5%, analytical grade), and distilled water.

- **Sample handling**

1. Cell culture medium samples: Take an appropriate amount of the sample, centrifuge at 1000 rpm for 10 minutes, and collect the supernatant for testing.
2. Serum (plasma) samples: use directly.
3. Tissue samples: Weigh the tissue sample, and add 9 volumes of normal saline at a mass (g) to volume (mL) ratio of 1:9. Mince the tissue thoroughly and prepare tissue homogenate in an ice water bath for subsequent detection.

   The homogenate can be centrifuged at 3500 rpm for 10 minutes, and the supernatant (10% tissue homogenate supernatant) is collected for measurement.

   For animal tissue homogenate supernatant, determine the protein concentration simultaneously and calculate the results according to Formula 3. For plant tissue homogenate supernatant, protein measurement is not required, and Formula 2 can be adopted for calculation.
4. Cell samples:

Cell collection: For suspension cultured cells: collect cell pellets directly by centrifugation (1000 rpm, 10 min); discard the supernatant and retain the cell precipitate. For adherent cultured cells: aspirate the culture supernatant, and scrape off cells directly with a cell scraper. Alternatively, digest cells with 0.25% trypsin at room temperature for 2-3 minutes, and terminate digestion with culture medium. Gently blow and mix the cell suspension with a pipette, transfer all liquid into an EP tube, centrifuge at 1000 rpm for 10 minutes, discard the supernatant and keep the cell pellet. Add 1 mL of PBS, pipette gently for resuspension, centrifuge again at 1000 rpm for 10 minutes, remove the supernatant, and retain the cell pellet for subsequent use.

Cell lysis (choose either method below): After lysis, the protein concentration can be determined using our BCA protein assay kit for subsequent calculation.

a. Grinding and lysis: Add an appropriate volume of buffer solution (PBS or normal saline is acceptable) to the cell pellet. For cells at the order of magnitude of 10^6, generally add 0.3-0.5 mL of buffer. Grind the mixture in an ice water bath with a manual glass homogenizer for 3-5 minutes, or use an electric tissue grinder for 3 minutes under ice-bath conditions, then set aside for testing.
b. Ultrasonic lysis: Add an appropriate volume of buffer solution (PBS or normal saline can be used) to the cell pellet. For cells at the order of magnitude of 10^6, generally add 0.3-0.5 mL of buffer. Ensure the ultrasonic probe is fully submerged below the liquid surface. Perform ultrasonication in an ice water bath with a power of 300 W; sonicate for 3-5 seconds each time at an interval of approximately 30 seconds, and repeat 3 to 5 times. (Recommended method)
c. Freeze-thaw cycles: Liquid nitrogen can be used for repeated freeze-thaw lysis. Add an

appropriate amount of hypotonic solution or distilled water to the cells in a cryotube, place the tube directly into liquid nitrogen for 3-5 seconds, then immediately transfer it to a -20°C refrigerator for 20-30 seconds, followed by thawing at room temperature. Repeat the above operation three times.

Note: Do not place the tube directly at room temperature immediately after taking it out of liquid nitrogen, as the rapid temperature change may cause the cryotube to crack and result in sample loss. Gradient thawing via low-temperature treatment is strictly required.

d. Chemical lysis: For adherent cultured cells, aspirate the supernatant directly, then add an appropriate amount of lysis buffer (1%-2% Triton X-100 is recommended, enough to fully cover the cells) directly into the culture plate or culture flask. Lyse the cells on ice for 30-40 minutes. The cell lysis status can be observed under a microscope; extend the lysis duration if the lysis effect is unsatisfactory. Finally, collect the lysate with a pipette for subsequent detection.

Operation process

1. Operation Procedure

	Blank tube	**Standard tube**	**Assay tube**	**Control tube**
10 nmol/mL Standard (mL)		0.2		
Anhydrous Ethanol (mL)	0.2			
Sample (mL)			0.2	0.2
Reagent 1 (mL)	0.2	0.2	0.2	0.2
Mix thoroughly				
Working Solution of Reagent 2 (mL)	3	3	3	3
Working Solution of Reagent 3 (mL)	1	1	1	
50% Glacial Acetic Acid (mL)				1

Cap the centrifuge tube tightly and mix thoroughly with a vortex mixer. Poke a small hole in the tube cap with a needle, or secure the cap tightly with an anti-explosion clamp without piercing. Heat the tubes in a boiling water bath above 95℃ for 40 minutes. After incubation, cool the tubes with tap water, then centrifuge at 4000 rpm at room temperature for 10 minutes. Collect the supernatant, set distilled water as the blank with a 1 cm optical path, and measure the absorbance (A) of each tube at 532 nm. Alternatively, transfer 0.2 mL of the reaction solution from each tube into a 96-well plate and read the absorbance at 532 nm using a microplate reader.

2. Simplified Operation Method

Preparation of Working Solution 1:
Reagent 1: Working Solution of Reagent 2: Working Solution of Reagent 3 = 0.2: 3: 1, prepare freshly before use.

Preparation of Working Solution 2:
Reagent 1: Working Solution of Reagent 2: Glacial Acetic Acid: Distilled Water = 0.2: 3: 0.5: 0.5, prepare freshly before use.

	Blank tube	**Standard tube**	**Assay tube**	**Control tube**
10 nmol/mL Standard (mL)		0.2		
Anhydrous Ethanol (mL)	0.2			
Sample (mL)			0.2	0.2
Working Solution 1 (mL)	4	4	4	
Working Solution 2 (mL)				4

Cap the centrifuge tube tightly and mix thoroughly with a vortex mixer. Poke a small hole in the tube cap with a needle, or secure the cap tightly with an anti-explosion clamp without piercing. Heat the tubes in a boiling water bath above 95℃ for 40 minutes. After incubation, cool the tubes with tap water, then centrifuge at 4000 rpm at room temperature for 10 minutes. Collect the supernatant, set distilled water as the blank with a 1 cm optical path, and measure the absorbance (A) of each tube at 532 nm. Alternatively, transfer 0.2 mL of the reaction solution from each tube into a 96-well plate and read the absorbance at 532 nm using a microplate reader.

Notes:

a. A control tube shall be set for each sample. All reagents in the table can be proportionally increased or decreased appropriately without affecting the final results.
b. The loading volume of cell homogenate or cell culture supernatant is 0.3-0.4 mL.
c. If the supernatant after post-reaction centrifugation is turbid due to high lipid content, add 0.1 mL of chloroform (analytical grade) to the reaction solution, vortex for 1 minute, and centrifuge again.
d. Reference absorbance of the standard tube: the absorbance value of the standard tube minus that of the blank tube is 0.130-0.140 (1 cm optical path); when detected by a microplate reader, the range is 0.075-0.090.
e. If the absorbance of test samples is too low in the pre-experiment, the water bath time can be extended from 40 minutes to 80 minutes in the formal experiment. To avoid inter-batch variation, all MDA water bath reactions in the same study must be performed for 80 minutes uniformly.

Calculation

1. Calculation Formula for MDA Content in Serum (Plasma) and Cell Culture Medium

$$\text{MDA Content } (\text{nmol/mL}) = \frac{A_{\mathbf{Assay}} - A_{\mathbf{Control}}}{A_{\mathbf{Standard}} - A_{\mathbf{Blank}}} \times C_{\mathbf{Standard}}$$

2. Calculation formula for MDA content in tissues by weight

$$\text{MDA Content } (\text{nmol/g}) = \frac{A_{\mathbf{Assay}} - A_{\mathbf{Control}}}{A_{\mathbf{Standard}} - A_{\mathbf{Blank}}} \times C_{\mathbf{Standard}} \div \frac{W}{V_{Total}}$$

3. Calculation Formula for MDA Content in Tissues (or Cells) Based on Protein Concentration

$$\text{MDA Content } (\text{nmol/mg}) = \frac{A_{\mathbf{Assay}} - A_{\mathbf{Control}}}{A_{\mathbf{Standard}} - A_{\mathbf{Blank}}} \times C_{\mathbf{Standard}} \div Cpr$$

4. Calculation Formula for MDA Content in Cells Based on Cell Number

$$\text{MDA Content } (\text{nmol}/10^6\text{cells}) = \frac{A_{\mathbf{Assay}} - A_{\mathbf{Control}}}{A_{\mathbf{Standard}} - A_{\mathbf{Blank}}} \times C_{\mathbf{Standard}} \div \frac{\text{Total cell number}}{V_{Total}}$$

Annotation:

V_{Total}: Total volume of normal saline added during tissue homogenization, mL

$C_{Standard}$: Standard concentration, 10 nmol/mL

Cpr: Protein concentration of homogenate (supernatant), mg/mL

W: Sample mass, g

Total cell number: refers to the total amount of cells collected before cell lysis, 10^6 cells.

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. Ensure thorough mixing during reagent preparation and before incubation in a 95℃ water bath.
4. The water bath time and temperature must be kept consistent. Laboratories without a water bath may substitute with boiling reactions using aluminum pots, boxes or basins.
5. For high-lipid samples, sufficient centrifugation and precipitation are required; otherwise, absorbance will be affected, leading to unstable results. In such cases, add 0.1-0.2 mL of chloroform to the reaction solution after the water bath, vortex thoroughly for 1-3 minutes, and then centrifuge again.
6. In winter, if the test solution appears cloudy, warm it gently in a water bath until it clarifies. Transfer the clear solution into a cuvette with a pipette. Persistent cloudiness indicates hyperlipidemia.
7. Sample loading volume: For samples in sufficient quantity or with low MDA content, the sample volume may be doubled. Meanwhile, double the dosage of distilled water, anhydrous ethanol and chloroform during extraction. For blood samples from anemic patients, the sample volume should also be doubled, while the amounts of distilled water, anhydrous ethanol and chloroform remain unchanged.
8. It is recommended to use covered test tubes or centrifuge tubes throughout the experiment to prevent evaporation of the reaction solution. If covered tubes are unavailable, seal the container tightly with refrigerator cling film secured by rubber bands, and pierce a small hole in the film with a needle as an alternative cover.