Technical Manual

Malondialdehyde (MDA) Colorimetric Assay Kit (Cell Samples)

- Catalogue Code: MAES0041
- Size: 500 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

500 Assays

Range:

0.29-100 nmol/mL

Sensitivity:

0.29 nmol/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

The body produce oxygen free radicals through the enzyme system and non-enzyme system, which can attack unsaturated fatty acid on biofilm and lead to lipid peroxidation and form lipid peroxide, such as aldehyde group (MDA), keto-, hydroxyl, carbonyl, etc. Oxygen free radicals cause cell damage not only by peroxidation of polyunsaturated fatty acids in biofilm, but also by decomposition products of lipid hydroperoxide. Detection of the MDA content can reflect the level of lipid peroxidation in cells and reflect level of cellular damage indirectly.

3. Intended Use

This kit can be used to measure the malondialdehyde (MDA) content in cell samples.

4. Detection Principle

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

5. Kit Components & Storage

Item	Specification	Storage
Clarificant	30 mL × 1 vial	2-8°C, 6 months
Acid Reagent	30 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	50 mL × 3 vial	2-8°C, 6 months, avoid direct sunlight
Standard (10 nmol/mL)	1.5 mL × 1 vial	2-8°C, 6 months
Extraction solution	30 mL × 1 vial	2-8°C, 6 months

Materials required but not supplied

- Micropipette
- Incubator
- Centrifuge
- Microplate Reader (530-540 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Absolute ethanol

6. Assay Notes:

1. It is recommended to fasten the glass tube mouth with preservative film and make a small hole in the film.

2. Water-bath temperature (95-100°C) and incubation time (40 min) should be stabilized. Cool the tubes with running water immediately once the incubation finished.

3. The supernatant for assay should not contain sediment, otherwise it will affect the OD values. It is recommended to use a pipette to take the supernatant.

4. Accurately take 250 μL reaction solution into the 96 wells microplate and without bubble.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **acid application solution:** Dilute the acid reagent with double distilled water at a ratio of 1.2: 34 and mix fully. The prepared solution can be stored at 2-8°C for 3 months.

3. **Preparation of working solution:** Mix the clarificant, acid application solution, chromogenic agent at a ratio of 0.2: 3: 1 fully. Prepare the fresh solution before use.

8. Sample Preparation

Cell sample:
Collect the cells into a centrifuge tube, add extraction solution at a ratio of cell number ($3*10^6$): extraction solution (μL) =1:300-500, mix fully for 2 min, then treat the cell with sonication (90W, 4s/time, interval for 2s, the total time is 10 min) or homogenization. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.29-100 nmol/mL).

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

Sample Type:	Dilution Factor:
A549 cells	1
HepG2 cells	1
293T cells	1

Note: The diluent is extraction solution;

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 532 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

Note: A, blank well; B, standard wells; S1-S92, sample wells.

10. Operation Steps

1. **Blank tube:** Take 0.1 mL of absolute ethanol (self-prepared) to the 1.5 mL EP tubes
   Standard tube: Take 0.1 mL of 10 nmol/mL standard to the 1.5 mL EP tubes.
   Sample tube: Take 0.1 mL of sample to the 1.5 mL EP tubes.

2. Add 1 mL of working solution into the wells of Step 1.

3. Mix fully with a vortex mixer. Tighten the tubes with preservative film and make a hole in the film. Incubate the tubes in 100°C water bath for 40 min.

4. Cool the tubes to room temperature with running water. Centrifuge at 1078 g for 10 min.

5. Take 250 μL of supernatant to microplate and measure the OD value at 532 nm with microplate reader.

Operation Table

	Blank tube	Standard tube	Sample tube
Absolute ethanol (self-prepared) (mL)	0.1		
Standard (mL) (10 nmol/mL)		0.1	
Sample (mL)			0.1
Working solution (mL)	1	1	1
Mix fully with a vortex mixer. Tighten the tubes with preservative film and make a hole in the film. Incubate the tubes in 100°C water baths for 40 min. Cool the tubes to room temperature with running water. Centrifuge at 1078 g for 10 min. Take 250 μL of supernatant to microplate and measure the OD value at 532 nm with microplate reader.			

11. Calculations

$$\text{MDA (nmol/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times C \times f \div C_{pr}$$

ΔA_1: $OD_{Sample} - OD_{Blank}$
ΔA_2: $OD_{Standard} - OD_{Blank}$
c: The concentration of standard, 10 nmol/mL
f: Dilution factor of sample before test
C_{pr}: Concentration of protein in sample, mgprot/mL

12. Performance Characteristics

Detection Range	0.29-100 nmol/mL
Sensitivity	0.29 nmol/mL
Average recovery rate (%)	95
Average inter-assay CV (%)	3.5
Average intra-assay CV (%)	3.3

Analysis

Take 0.1 mL of HepG2 cell homogenate, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.068, the average OD value of the blank is 0.043, the average OD value of the standard is 0.211, the concentration of protein in sample is 3.38 mgprot/mL, and the calculation result is:

$$\text{MDA (nmol/mgprot)} = \frac{0.068-0.043}{0.211-0.043} \times 10 \div 3.38$$

$$= 0.44 \text{ nmol/mgprot}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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