ISO 13485:2016ISO 9001:2015

Ver.240816

Malondialdehyde (MDA) Assay Kit

BC2202-01 (50 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Lipid peroxide is produced by the action of oxygen free radicals on unsaturated fatty acid, then resolves to compounds, including malondialdehyde (MDA). The level of lipid peroxidation can be showed by detecting the level of MDA.

Under acidic and high temperature conditions, the brownish red 3,5,5- three methyl sulfamethoxazole -2,4-two ketone is synthesized with MDA and thiobarbituric acid (TBA) taking place condensation reaction, and the largest absorption wavelength is 532 nm. After colorimetry, the MDA content in the sample can be estimated.

Kit components

Reagent	Volume	Storage
Extraction Reagent	60 mL × 1	2-8°C
Reagent I	42 mL× 1	2-8°C
Reagent II	Powder × 2	2-8°C
MDA Working Reagent: Add 20 mL of Reagent I to each Reagent II, dissolve and mix thoroughly. Storage at 2-8℃ for one month. The working solution for MDA detection is difficult to dissolve, which can be heated at 70℃ and vibrated violently to promote dissolution. Or by ultrasonic treatment to promote dissolution		
Reagent III	12 mL	2-8°C

Reagents and Equipment Required but Not Provided

Spectrophotometer, water bath, desk centrifuge, transferpettor, mortar/homogenizer/cell ultrasonic crusher, 1 mL glass cuvette, ice and distilled water.

Sample Preparation

Bacteria or cells:

Collect bacteria or cells into the centrifuge tube. 5 million bacteria or cells could be mixed with 1 mL of Extraction reagent. Use ultrasonication to split bacteria and cells (placed on ice, ultrasonic power 200W, ultrasonic time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take supernatant on ice before testing.

Tissue:

0.1 g of tissue could be mixed with 1 mL of Extraction reagent and fully homogenized on ice bath. Then centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

Serum:

Detect directly

Samples with high fat content:
Add 40 μL sample to 80 μL ethanol (dilute sample three times with ethanol), mix for 5 minutes. The appropriate dilution ratio of the sample was determined by pre-experiment. And replace 200μL distilled water of blank tube with 66μL distilled water and 134μL ethanol.

Determination Procedures

1. Preheat the spectrophotometer for more than 30 minutes and set zero with distilled water.
2. Add reagents with the following list:

Reagent(μL)	Test tube (T)	Blank tube (B)
MDA working reagent	600	600
Sample	200	-
Distilled water	-	200
Reagent III	200	200

The mixture would be incubated at 100℃ for 60 minutes (tightly close to prevent moisture loss), cooled on ice, and centrifuged at 10000 ×g for 10 minutes at room temperature to remove insoluble materials. Take supernatant in 1 mL glass cuvette and measure the absorbance at 532 nm and 600 nm, ΔA532=A532 (T)-A532 (B), ΔA600 =A600 (T)-A600 (B), ΔA=ΔA532-ΔA600. Blank tube needs to test once or twice

Note1: Replace 200μL distilled water of blank tube with 66μL distilled water and 134μL ethanol for Samples with high fat content;
Note2: Be careful during the reaction in a boiling water bath. It is recommended to use EP tubes with screw caps. Or use a needle to pierce a small hole on the lid of tube with snap cap to prevent the lid from bursting. The tube cap could be pressed by heavy objects if heated by using metal bath.

Calculations

1. **Protein concentration**

$$\text{MDA content (nmol/mg prot)} = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (Cpr \times Vs) \times F$$
$$= 32.258 \times \Delta A \div Cpr \times F$$

2. **Sample weight**

$$\text{MDA content (nmol/g weight)} = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (W \times Vs \div Vsv) \times F$$
$$= 32.258 \times \Delta A \div W \times F$$

3. **Cell amount**

$$\text{MDA content (nmol/10}^4\text{cell)} = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (500 \times Vs \div Vsv) \times F$$
$$= 0.0645 \times \Delta A \times F$$

4. **Serum volume**

$$\text{MDA content (nmol/mL)} = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div Vs \times F$$
$$= 32.258 \times \Delta A \times F$$

Vrv: Total reaction volume, 0.001L

ε: Molar extinction coefficient, 1.55×10^{5} L/mol/cm

d: light path of cuvette,1cm

Vs: Sample volume, 0.2 mL

Vsv: Extraction volume, 1 mL

Cpr: Sample protein concentration, mg/mL;

W : Sample weight, g;

500: Total number of bacteria and cells, 5 million.

F: dilution factor

Note:

If it is found that the absorbance value of the sample is too low, the boiling water bath time can be adjusted from 60 minutes to 90 minutes or longer. The detection of MDA in the same experiment needs to be extended to the same time to avoid errors.