

Ver.250501

Malondialdehyde (MDA) Activity 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, which then resolves into compounds including malondialdehyde (MDA). The level of lipid peroxidation can be showed by detecting the level of MDA.

In the lipid peroxidation assay protocol, the MDA in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct. The MDA-TBA adduct can be easily quantified colorimetrically (OD = 532 nm) or fluorometrically (Ex/Em = 532/553 nm). Presence of soluble sugars affects the assay by reacting with TBA to give a product with absorption at 450nm and 532nm. In this kit, the MDA content is calculated by difference between the absorbance at 532nm, 450nm, and 600nm.

The kit has two formulas which takes into consideration the presence of sucrose and glucose.

Kit components

Reagent	Volume	Storage
Extraction Reagent	60mL	4°C
Reagent I	42mL	4°C
Reagent II	Powder × 2	4°C
MDA Working reagent: Add 20mL Reagent I to each Reagent II, dissolve (heat at 70°C with thorough mixing). Working Reagent can be stored at 4°C for 1 month		
Reagent III	12mL	4°C

Note: The working solution for MDA detection is difficult to dissolve, which can be heated at 70°C and vortexed or ultrasonicated to completely dissolve.

Reagents and Equipment Required but Not Provided

Constant temperature water bath, desktop centrifuge, spectrophotometer, 1mL glass cuvette, ice homogenizer and distilled water.

Sample Preparation

Bacteria or cells:

Collect 5×10^6 to centrifuge tube and add 1mL of Extraction Reagent. Use ultrasonication for splitting the bacteria and cells (to be performed on ice, power 20%, 3 seconds with 10 seconds interval, repeat 30 times). Centrifuge at $8000 \times g$ for 10 minutes at 4°C to remove the insoluble materials. Transfer the supernatant into a new tube and place on ice.

Tissue:

Add 1mL Extraction reagent to 0.1g tissue and fully homogenize on ice bath. Centrifuge at $8000 \times g$ for 10 minutes at 4°C to remove the insoluble materials. Transfer the supernatant into a new tube and place on ice.

Serum:

Use directly

Samples with fat content:

Add 40μL sample to 80μL ethanol (dilute sample three times with ethanol), mix for 5 minutes. The appropriate dilution ratio of the sample was determined by pre-experiment. Blank tube contains 66μL distilled water and 134μL ethanol.

Operation Procedures

1. Preheat the spectrophotometer/microplate reader for 30 minutes and set zero with distilled water.
2. Add reagents to 1.5mL tube as follows

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

3. Place the mixture in a boiling waterbath (100°C) for 60 minutes (keep the tubes tightly closed to prevent evaporation). Cool by placing on ice and centrifuge at 10000 ×g for 10 minutes to remove insoluble materials.
4. Transfer supernatant to 1mL glass cuvette and measure absorbance at 450nm, 532nm and 600nm

$$\Delta A_{450} = A_{450T} - A_{450B}$$

$$\Delta A_{532} = A_{532T} - A_{532B}$$

$$\Delta A_{600} = A_{600T} - A_{600B}$$

Note:

1. Replace 200μL distilled water of blank tube with 66μL distilled water and 134μL ethanol for Samples with high fat content;
2. 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.

Calculations

Tissue, bacteria and cultured cells

Protein concentration:

$$\begin{aligned} \text{MDA (nmol/mg prot)} &= [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (C_{pr} \times V_s) \times F \\ &= 32.258 \times \Delta A \div C_{pr} \times F \end{aligned}$$

Sample weight:

$$\begin{aligned} \text{MDA (nmol/g weight)} &= [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (W \times V_s \div V_{sv}) \times F \\ &= 32.258 \times \Delta A \div W \times F \end{aligned}$$

Cell amount:

$$\begin{aligned} \text{MDA (nmol/10}^4 \text{ cell)} &= [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (500 \times V_s \div V_{sv}) \times F \\ &= 0.0645 \times \Delta A \times F \end{aligned}$$

Serum:

$$\begin{aligned} \text{MDA (nmol/mL)} &= [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div V_s \times F \\ &= 32.258 \times \Delta A \times F \end{aligned}$$

V_{rv} : Total reaction volume, 0.001L

V_s : Sample volume, 0.2mL

V_{sv} : Extraction volume, 1mL

C_{pr} : Sample protein concentration, mg/mL

W : Sample weight, g

500 : Total number of bacteria and cells, 5 million

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

d : light path of cuvette, 1cm

F : Dilution factor