Ver.P261001

Malondialdehyde (MDA) Activity Assay Kit

BC2202-02 (100S)

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 = 532nm) or fluorometrically (Ex/Em = 532/553nm). Absorbance of sugar is measured at 450nm.

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

Kit components

Reagent	Volume	Storage
Extraction Reagent	110mL	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×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×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
   Note: A single blank reaction is sufficient for each set of assays.

Reagent	Test (T)	Blank (B)
MDA working reagent	300μL	300μL
Sample	100μL	-
Distilled water	-	100μL
Reagent III	100μL	100μL

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 to1mL glass cuvette and measure absorbance at 532nm.

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.
3. If sample has high sugar content, measure OD at 450nm also and subtract the value from OD 532nm before proceeding to calculation.
   Calculation will become = ΔA532 – ΔA450

Calculations

Tissue, bacteria and cultured cells

Protein concentration:

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

Sample weight:

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

Cell amount:

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

Serum:

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

Vrv : Total reaction volume, 0.001L
Vs : Sample volume, 0.2mL
Vsv : Extraction volume, 1mL
Cpr : 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