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Ver.250201

## **Malondialdehyde (MDA) Activity Assay Kit**

BC2202-02 (100 Tests/96 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	110mL	4°C
Reagent I	42mL	4°C
Reagent II	Powder × 1	4°C
MDA Working reagent: Add 40mL Reagent I to Reagent II, dissolve (heat at 70°C or by ultrasonication. Store at 4°C.		
Reagent III	12mL	4°C
Note: The working solution for MDA detection is difficult to dissolve. Heat at 70°C and vortex thoroughly to promote dissolution.		

## Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer / microplate reader, micro glass cuvette / 96-well flat bottom plates and distilled water.

## Sample Preparation

### *Bacteria or cells:*

Collect  $5 \times 10^6$  to centrifuge tube and add 1mL of Extraction Reagent. Use ultrasonication for splitting the the bacteria and cells (to be performed on ice, power 20%, 3 seconds with 10 second 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

## Operation Procedures

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

Reagent	Test (T)	Blank (B)
MDA working reagent	300 $\mu$ L	300 $\mu$ L
Sample	100 $\mu$ L	-
Distilled water	-	100 $\mu$ L
Reagent III	100 $\mu$ L	100 $\mu$ L

3. Place the mixture in a boiling water bath (100°C) for 60 minutes (keep the tubes tightly closed to prevent evaporation). Cool by placing on ice and centrifuge at 10000  $\times g$  for 10 minutes to remove insoluble materials.
4. Transfer 200 $\mu$ L supernatant in a glass cuvette and measure absorbance at 532nm and 600nm

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

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

$$\Delta A = \Delta A_{532} - \Delta A_{600}$$

Note: Blank Tube needs to be done in duplicates.

If the absorbance is low, boiling time can be increased to 90 minutes. All the samples and blank in the batch should be treated the same to avoid errors.

## Calculations

### 1. 96 Well Plate:

*Protein concentration:*

$$\begin{aligned} \text{MDA (nmol/mg prot)} &= [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (C_{pr} \times V_s) \\ &= 53.763 \times \Delta A \div C_{pr} \end{aligned}$$

*Sample weight:*

$$\begin{aligned} \text{MDA (nmol/g weight)} &= [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (W \times V_s \div V_{sv}) \\ &= 53.763 \times \Delta A \div W \end{aligned}$$

*Cell amount:*

$$\begin{aligned} \text{MDA (nmol/10}^4 \text{ cell)} &= [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div (500 \times V_s \div V_{sv}) \\ &= 0.1075 \times \Delta A \end{aligned}$$

*Serum:*

$$\begin{aligned} \text{MDA (nmol/ml)} &= [\Delta A \times V_{rv} \div (\epsilon \times d) \times 10^9] \div V_s \\ &= 53.763 \times \Delta A \end{aligned}$$

$V_{rv}$  : Total reaction volume,  $5 \times 10^{-4}$ L

$\epsilon$  : Molar extinction coefficient,  $1.55 \times 10^5$  L/mol/cm

$d$  : Light path of 96 well plate, 0.6cm

$V_s$  : Sample volume, 0.1mL

$V_{sv}$  : Volume of Extraction Reagent, 1mL

$C_{pr}$  : Sample protein concentration (mg/ml)

$W$  : Sample weight in grams.

500 : Total number of cells, 5 million

## 2. *Micro glass cuvette:*

*Protein concentration:*

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

*Sample weight:*

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

*Cell amount:*

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

*Serum:*

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

$V_{rv}$  : Total reaction volume,  $5 \times 10^{-4}\text{L}$

$\epsilon$  : Molar extinction coefficient,  $1.55 \times 10^5 \text{ L/mol/cm}$

$d$  : Light path of micro glass cuvette, 1cm

$V_s$  : Sample volume, 0.1mL

$V_{sv}$  : Volume of Extraction Reagent, 1mL

$C_{pr}$  : Sample protein concentration (mg/ml)

$W$  : Sample weight in grams.

500 : Total number of cells, 5 million