



# Cell Malondialdehyde (MDA) assay kit

(Cat/No.:BC129 Size:400T)

## 1. Compositions and Preparation (The kit is valid for 1 year)

|   | Format          | Storage Condition(s) | Term of Validity |
|---|-----------------|----------------------|------------------|
| Reagent I   | 1 Bottle×25 ml  | Room Temperature     | 1 Year           |
| Were the temperature too low, reagent I may be frozen and under such circumstance, the reagent can be warmed in a water bath to accelerate thawing till transparent before use. |                 |                      |                  |
| Reagent II  | 1 Bottle×12 ml  | 4°C                  | 1 Year           |
| Preparation of Reagent II Solution: Dilute the reagent II with 340 ml double distilled water (DDW) before the measurement, rest can be preserved at 4°C.                        |                 |                      |                  |
| Reagent III   | 2 Bottles×60 ml | 4°C, Avoid Light     | 1 Year           |
| Preparation of the Working Fluid: Blend reagent I, II solution and III with the ratio of 0.2:3:1 (v/v) prior to the measurement with the exact amount needed.                   |                 |                      |                  |
| Reagent IV  | 1 Vial×1 ml     | 4°C, Sealed          | 1 Year           |
| Reagent V   | 1 Bottle×250 ml | 4°C, Sealed          | 1 Year           |

## 2. Assay Procedures

### a、 Pretreatment

Centrifuge the sample if necessary and discard the culture supernatant. Collect the cells precipitated and transfer to a centrifuge tube. Add 0.5 ml reagent V and mix for 2 min. Disrupt the cells (homogenize or sonicate the mixture) and transfer 0.1 ml resulted suspension into another 1.5 ml capped centrifuge tube (make a hole with needles on the cap).

### b、 Measurement

| Components (μl)   | Blank | Standard | Sample |
|-------------------|-------|----------|--------|
| Absolute Ethanol  | 100   |          |        |
| Reagent IV        |       | 100      |        |
| Pretreated Sample |       |          | 100    |
| Working Fluid     | 1,000 | 1,000    | 1,000  |

Cap the tube and vortex the mixture thoroughly. Heat the mixture in a boiled water bath for 40 min and cool with water. Centrifuge at 4,000 rpm for 10 min. Zero the microplate at 530 nm and transfer 0.25 ml of the supernatant to a well. Record the optical density (OD) value of each well.



### 3. Calculation Formula

$$\frac{MDA\ Conc.}{mgprot} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{mL} \div \frac{C_{Protein}}{mgprot}$$

### 4. Example

A sample was treated and measured with the OD values equal to 0.002, 0.266 and 0.094 respectively. The protein concentration of pre-treated sample supernatant was 0.144 mgprot/ml.

$$\begin{aligned} \frac{MDA\ Conc.}{mgprot} &= \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{mL} \div \frac{C_{Protein}}{mgprot} \\ &= \frac{0.094 - 0.002}{0.266 - 0.002} \times \frac{10nmol}{mL} \div \frac{0.144mgprot}{mL} = \frac{24.2nmol}{mgprot} \end{aligned}$$

### 5. Principle of the Assay

Thiobarbituric acid can react with MDA to generate red compound which has the maximum absorbance at 532nm. Such assay is commonly called as thiobarbituric acid reactive substances assay (TBARS assay).

### 6. Significance of the Assay

Enzymatic or non-enzymatic reactions in vivo produce oxygen radicals which may attack the poly-unsaturated fatty acid (PUFA) in membranes which is commonly known as lipid peroxidation. The end products of lipid peroxidation are active aldehydes such as MDA and 4-hydroxynonenal (HNE). Not only would such process transfer oxygen radicals to other radicals, but increase the radical activity in vivo through propagation as well. Among end product of lipid peroxidation, some are harmless while others may cause dysfunction or dysbolism of cells or even death. Both oxygen radicals and end products of lipid peroxidation may cause damage to cells. Thus the MDA level in vivo can represent the lipid peroxidation extent and indirectly represent the degree of cell damage.

MDA assay is commonly accomplished with Superoxide Dismutase, Xanthine Oxidase, Nitric Oxide, Total-Antioxidant Capacity, Monoamine Oxidase, Peroxidase, Lipofuscin, Free Fatty Acid, Lipase, Total Lipase, Triglyceride, Total Cholesterol, Low Density Lipoprotein and High Density Lipoprotein assays and will represent the ability to eliminate oxygen radicals in vivo and the severity of radical attacks.