# Malondialdehyde (MDA) Assay Kit

**Cat No. MDA-M-50**
Size: 50 Reactions
Store at 4°C

MOLEQULE-ON®

| Components | Quantity |
|---|---|
| Extraction Reagent | 50 ml |
| Reagent I | 30 ml |
| Reagent II | 2 vials |
| Reagent III | 10 ml |

## 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 brown red 3,5,5- three methyl sulfamethoxazole -2,4-two ketone was synthesized with MDA and thiobarbituric acid (TBA) taking place condensation reaction, the largest absorption wavelength is 532 nm. The content of lipid peroxidation can be estimated after colorimetric. But the soluble sugar will disturb the detection, the production (color reaction of soluble sugar with TBA) have absorption wavelength in 450 nm and 532 nm. In this kit, the MDA content is calculated by the difference between the absorbance at 532 nm, 450 nm and 600 nm. Due to the effect of sucrose in plant tissues and glucose in animal tissues, this kit have two computational formulas for sucrose and glucose.

## Reagents and Equipment Required but Not Provided:

Spectrophotometer, benchtop centrifuge, micropipette, cuvette, mortar, ice and distilled water.

## Preparation:

**MDA working reagent:** add 15 ml reagent I to reagent Ⅱ, dissolve (heat at 70℃ or with ultrasonic) and mix.

## Protocol

### I. Sample preparation:

**1. Bacteria or cells:**
Collect bacteria or cells into the centrifuge tube, suggested is 4 million cells with 1mL Extraction reagent. Use ultrasonication to split bacteria and cell (placed on ice, 200W, work time 3s，interval 10s，repeat for 30 times). Centrifuge at 8000 rpm for 10 minutes at 4℃. Supernatant is used for test.
Suggested 0.1g tissue with 1mL Extraction reagent. Fully grinding on ice, centrifuge at 8000 rpm for 10 minutes at 4℃. Supernatant is used for test.

**2. Tissue sample:**
Suggested 0.1 g tissue with 1 mL Extraction reagent and fully homogenized on ice bath. Centrifuge at 8000 rpm for 10 minutes at 4℃ to separate the layers, and take the supernatant on ice before testing.

**3. Serum**
Detect directly

### II. Determination procedure:

1. Preheat the spectrophotometer for more than 30 minutes, set zero with distilled water.

2. Add reagents with the following list:

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

3 Incubate the mixture at 100℃ for 30 minutes. Cool in an ice bath. Centrifuge at 10000 rpm for 10 minutes at room temperature to remove insoluble materials. Take supernatant in 1 mL cuvette, measure the absorbance at 450 nm, 532 nm and 600 nm. Calculate ΔA450 = A450(T)-A450(B), ΔA532 = A532(T)-A532(B), ΔA600 = A600(T)-A600(B).

## Calculations

**1. Tissue, bacteria or cultured cells**

**A. Protein concentration:**
MDA(nmol/ mg prot)=(12.9×(ΔA532-ΔA600)-2.58×ΔA450)×Vrv÷（Cpr×Vs）
=5×(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)÷Cpr

**B. Sample weight:**
MDA(nmol/g)=(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)×Vrv÷（W×Vs÷Vsv）
=5×(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)÷W

**C. Cell amount:**
MDA(nmol/$10^4$cell)=(12.9 ×(ΔA532-ΔA600)-2.58×AΔ450)×Vrv÷(400×Vs÷Vsv)
=0.125×(12.9 ×(ΔA532-ΔA600)-2.58×ΔA450)

**2. Plant:**

**A. Sample weight**
MDA(nmol/ g)=(12.9 ×(ΔA532-ΔA600)-1.12×ΔA450)×Vrv÷(W×Vs÷Vsv)
=5×(12.9 ×(ΔA532-ΔA600)-1.12×ΔA450)÷W

**B. Protein concentration:**
MDA(nmol/ mg prot)=(12.9 ×(ΔA532-ΔA600)-1.12×ΔA450)×Vrv÷(Cpr×Vs)
=5×(12.9 ×(ΔA532-ΔA600)-1.12×ΔA450)÷Cpr.

**3. Serum**
MDA(nmol/ ml)= (ΔA x Vrv) ÷ (ε x d) x $10^9$] ÷ Vs = 32.258 x ΔA

Vrv: total reaction volume, 1 mL;
ε: Molar extinction coefficient, 1.55x$10^5$ L/mol/cm
d: light path of 96-well plate, 0.6cm
Vs: sample volume,0.2 mL;
Vsv: extraction volume, 1 mL;
Cpr: Sample protein concentration, mg/mL;
W: sample weight, g;
400: total number of bacteria and cells, 4 million.