Instruction Manual

Malondialdehyde Assay Kit (Microanalysis)

Description

Malondialdehyde (MDA), a terminal product of lipid peroxidation, affects mitochondrial respiratory chain complexes and the activities of key mitochondrial enzymes in vitro. MDA is one of the most important products of membrane lipid peroxidation, and its formation further aggravates membrane damage. Therefore, in studies of plant senescence physiology and stress resistance physiology, MDA content is commonly used as an indicator. Measuring MDA allows assessment of the extent of membrane lipid peroxidation, thereby indirectly evaluating the degree of damage to membrane systems and the stress tolerance of plants.

Detection Principle

• Rich in surface-active functional groups: Magrose features a high density of COOH groups (~1000 μmol/g), ensuring efficient target binding and extremely low nonspecific adsorption.

• Uniform magnetic bead dispersion with good operability, maintaining stable magnetic responsiveness and excellent resuspension performance.

• Excellent physicochemical stability and good batch-to-batch reproducibility; the coefficient of variation (CV) of amino group content between batches is <5%, ensuring stable and reliable experimental results.

• High target binding capacity with low nonspecific adsorption, specifically designed for separation and purification applications.

Packing

Taking 100T/96S packing for example:

Components	Packing	Storage
CB0094M-ES	100mL×1	4 ℃
CB0094M-A	30mL×1	4 ℃
Before use, check whether CB0094M-A is completely dissolved. If not fully dissolved, heat to 70 °C and vortex to facilitate dissolution.		
Before the formal assay, perform a pilot test using 2–3 samples with expected large differences.		

Instructions

1. Preparation of Lab Instruments

Visible spectrophotometer / microplate reader, micro glass cuvettes / 96-well plates, water bath, benchtop centrifuge, adjustable micropipettes, mortar, ice, and distilled water.

2. MDA Extraction Solution

1) Preparation of bacterial and cell samples:

Bacteria or cultured cells: Collect the bacteria or cells into centrifuge tubes. After centrifugation, discard the supernatant. Add CB0094M-ES according to the ratio of bacterial or cell number (10^4 cells): CB0094M-ES volume (mL) = 500~1000 : 1 (recommended: add 1 mL of CB0094M-ES to 5 x 10^6 bacteria or cells). Lyse the bacteria or cells by ultrasonication (on ice; 20% power or 200 W; sonicate for 3 s with 10 s intervals, repeat 30 times). Centrifuge at 8000 × g for 10 min at 4 °C. Collect the supernatant and keep it on ice for analysis.

2) Preparation of tissue samples:

Add CB0094M-ES according to the ratio of tissue weight (g): CB0094M-ES volume (mL) = 1:5~10 (recommended: weigh approximately 0.1 g of tissue and add 1 mL of CB0094M-ES), then homogenize in an ice bath. Centrifuge at 8000 × g for 10 min at 4 °C. Collect the supernatant and keep it on ice for analysis.

3) Serum (plasma) samples: Analyze directly.

3. Assay Procedure

1) Pipette 0.3 mL of CB0094M-A into a 1.5 mL centrifuge tube, then add 0.1 mL of the sample and mix well.

2) Incubate in a 95 °C water bath for 30 min (cap tightly to prevent moisture loss), cool in an ice bath, then centrifuge at 10,000 × g, 25 °C for 10 min.

3) Transfer 200 μL of the supernatant to a micro glass cuvette or 96-well plate, measure the absorbance at 532 nm and 600 nm, record as A532 and A600. ΔA = A532 − A600.

4. Calculation of MDA Content

a. The calculation formula for measurements using micro glass cuvettes:

1. Calculation of MDA content in serum (plasma)

MDA content (nmol/ mL) = [ΔA×V1÷(ε×d)×109]÷V2 = 25.8×ΔA

2. Calculation of MDA Content in Bacteria, Cells, or Animal Tissues

(1) Based on protein concentration

MDAcontent (nmol/ mg prot) = [ΔA×V1÷(ε×d)×109]÷(Cpr×V2) = 25.8× ΔA ÷Cpr

(2) Based on sample mass

MDA content (nmol/g fresh sample weight) = [ΔA×V1÷(ε×d)×109]÷(W ×V2÷V3) = 25.8×ΔA ÷W

(3) Based on bacterial or cell density

MDA content (nmol/104) = [ΔA×V1÷(ε×d)×109]÷(500×V2÷V3) = 0.0516×ΔA

Note: V1：total reaction volume， 4×10-4 L；

ε：molar extinction coefficient of malondialdehyde (MDA)，155×103 L/mol/cm；

d：optical path length of the cuvette，1cm；

V2：volume of sample added，0.1 mL；

V3：volume of extraction solution added，1 mL；

Cpr：sample protein concentration，mg/mL；

W：sample mass，g；

500：total number of cells or bacteria，500 million.

b. The calculation formula for measurements using a 96-well plate:

1. Calculation of MDA content in serum (plasma)

MDA content (nmol/ mL) = [ΔA×V1÷(ε×d)×109]÷V2 = 51.6×ΔA

2. Calculation of MDA Content in Bacteria, Cells, or Animal Tissues

(1) Based on protein concentration

MDA content (nmol/ mg prot) = [ΔA×V1÷(ε×d)×109]÷(Cpr×V2) = 51.6×ΔA÷Cpr

(2) Based on sample mass

MDA content (nmol/g fresh sample weight) = [ΔA×V1÷(ε×d)×109]÷(W ×V2÷V3) = 51.6×ΔA ÷W

(3) Base on bacterial or cell density

MDA content (nmol/104) = [ΔA×V1÷(ε×d)×109]÷(500×V2÷V3) = 0.1032×ΔA

Note: V1：total reaction volume，4×10-4 L；

ε：molar extinction coefficient of malondialdehyde (MDA)，155×103 L/mol/cm；

d：Optical path length of a 96-well plate，0.5cm；

V2：volume of sample added，0.1 mL；

V3：volume of extraction solution added，1 mL；
Cpr：sample protein concentration，mg/mL；
W：sample mass，g；
500：total number of cells or bacteria，500 million.

Precautions

1. For protein quantification, it is recommended to use BCA Protein Quantification Kit (C0050) .
2. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
3. Please wear a lab coat and disposable gloves.

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