Malondialdehyde (MDA) Content Assay Kit

Cat. No. KITG-077 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Malondialdehyde (MDA) is an end product of lipid peroxidation in organisms. MDA can cause cross-linking and polymerization of biological macromolecules such as proteins and nucleic acids and is cytotoxic. Its content is an important parameter reflecting the potential antioxidant capacity of an organism, the rate and intensity of lipid peroxidation, and indirectly, the degree of tissue peroxidative damage. Under acidic and high-temperature conditions, MDA reacts with thiobarbituric acid (TBA) to form a red trimethine product (3,5,5-trimethyloxazolidine-2,4-dione), which has a characteristic absorption peak at 532 nm. MDA content can therefore be quantified from the change in absorbance. When MDA is measured in animal or plant tissues, several substances may interfere, with soluble sugars being the principal interferents. Soluble sugars also react with TBA to produce color and absorb at both 532 nm and 450 nm, with the product maximum generally occurring at 450 nm. Absorbance must therefore be measured simultaneously at 600 nm, 532 nm, and 450 nm, and the differences among the absorbance values at these wavelengths are used to accurately quantify MDA content. The kit includes specialized reagents and procedures for removing macromolecular interferents from samples. These measures effectively prevent the trimethine product from forming complexes with macromolecules and being lost in the precipitate, thereby reducing error and helping ensure accurate results.
Description	Method: Visible spectrophotometry. Pack Size: 60 tests / 50 samples. For Research Use Only. Not for Use in Diagnostic Procedures.
Form	Kit

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Applications	Quantitative determination of malondialdehyde (MDA) content in animal tissues, plant tissues, bacteria, cells, serum, plasma, culture medium, and other liquid samples.
Notes	Instructions for Use Required Instruments and Reagents: Visible spectrophotometer; 1 mL glass cuvette with a 10 mm optical path, 3 mm slit, and 1.05 mL volume; mortar or homogenizer; adjustable pipette; benchtop centrifuge; tightly sealing screw-cap centrifuge tubes (cryovials are recommended); sealing film; thermostatic water bath or incubator; and distilled water. 1. Sample Preparation The sample amount and extraction ratio may be adjusted appropriately according to preliminary experimental results. 1) Tissue: Process the sample at a tissue mass (g) to Extraction Solution volume (mL) ratio of 1:(5-10). A suggested procedure is to weigh 0.1 g tissue and add 1 mL Extraction Solution. Homogenize in an ice bath. Centrifuge at 8,000 × g for 10 min at 4°C. Collect the supernatant and keep it on ice for analysis. 2) Bacteria or cells: Collect bacteria or cells by centrifugation. Process the sample at a bacterial or cell number (10^4) to Extraction Solution volume (mL) ratio of (500-1,000):1. A suggested procedure is to add 1 mL Extraction Solution to 5 × 10^6 bacteria or cells. Disrupt the bacteria or cells by sonication in an ice bath at 200 W using 3 s sonication and 10 s intervals for 30 cycles. Centrifuge at 8,000 × g for 10 min at 4°C. Collect the supernatant and keep it on ice for analysis. 3) Serum, plasma, culture medium, and other liquid samples: Analyze directly or after appropriate dilution. 2. Assay Procedure 1) Preheat the spectrophotometer for at least 30 min. Set the wavelengths to 450 nm, 532 nm, and 600 nm, respectively, and zero the instrument with distilled water at each wavelength. 2) Add the following reagents sequentially to tightly sealing screw-cap centrifuge tubes: Assay tube: 300 µL sample and 100 µL Reagent 1. Blank tube: 300 µL distilled water and 100 µL Reagent 1. Mix thoroughly, heat at 100°C for 5 min, and cool to room temperature in an ice bath. Centrifuge at 8,000 × g for 10 min at room temperature and collect the supernatant. For both the assay and blank tubes, combine 300 µL of the corresponding supernatant with 500 µL Detection Working Solution and 200 µL

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Reagent 3. Mix thoroughly, heat at 100°C for 60 min, and cool to room temperature in an ice bath. Centrifuge at 8,000 × g for 10 min at room temperature and collect the supernatant. The 100°C treatment is performed at high temperature for an extended period. Seal the tubes carefully to prevent water loss. 3) Absorbance Measurement: Transfer the final supernatant to a 1 mL glass cuvette and measure absorbance at 450 nm, 532 nm, and 600 nm. Calculate $\Delta A_{450} = A_{450}$, assay - A_{450}, blank; $\Delta A_{532} = A_{532}$, assay - A_{532}, blank; and $\Delta A_{600} = A_{600}$, assay - A_{600}, blank. The blank tube only needs to be measured 1-2 times. 3. Calculation of MDA Content 3.1 MDA Content in Bacteria, Cells, or Animal Tissues 1) Based on animal tissue protein concentration: MDA content (nmol/mg protein) = $[6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}] \times V_1 \times (V_2 / V_3) / V_5 / Cpr \times D = 4.44 \times [6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}] / Cpr \times D$. 2) Based on animal tissue mass: MDA content (nmol/g) = $[6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}] \times V_1 \times (V_2 / V_3) \times (V_4 / V_5) / W \times D = 4.44 \times [6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}] / W \times D$. 3) Based on bacterial or cell number: MDA content (nmol/10^4 cells) = $[6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}] \times V_1 \times (V_2 / V_3) \times (V_4 / V_5) / cell \times D = 4.44 \times [6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}] / cell \times D$. 4) Based on liquid sample volume: MDA content (nmol/mL) = $[6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}] \times V_1 \times (V_2 / V_3) / V_5 \times D = 4.44 \times [6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}] \times D$. 3.2 MDA Content in Plant Tissues 1) Based on plant tissue protein concentration: MDA content (nmol/mg protein) = $[6.45 \times (\Delta A_{532} - \Delta A_{600}) - 0.56 \times \Delta A_{450}] \times V_1 \times (V_2 / V_3) / V_5 / Cpr \times D = 4.44 \times [6.45 \times (\Delta A_{532} - \Delta A_{600}) - 0.56 \times \Delta A_{450}] / Cpr \times D$. 2) Based on plant tissue mass: MDA content (nmol/g) = $[6.45 \times (\Delta A_{532} - \Delta A_{600}) - 0.56 \times \Delta A_{450}] \times V_1 \times (V_2 / V_3) \times (V_4 / V_5) / W \times D = 4.44 \times [6.45 \times (\Delta A_{532} - \Delta A_{600}) - 0.56 \times \Delta A_{450}] / W \times D$. Definitions: V_1, total color-development system volume (supernatant + Detection Working Solution + Reagent 3), 1 mL; V_2, total first-reaction volume (sample + Reagent 1), 0.4 mL; V_3, volume of supernatant added to the color-development system, 0.3 mL; V_4, total sample extract volume, 1 mL; V_5, sample volume added to the first reaction system, 0.3 mL; Cpr, protein concentration of the sample,

mg/mL; W, sample mass, g; cell, bacterial or cell number expressed in units of 10^4, so 5×10^6 bacteria or cells is entered as 500; D, dilution factor of the sample, entered as 1 when the sample is not diluted. 4. Precautions 1) The red trimethine product formed after the reaction may form complexes with macromolecules in the sample. After the final 100°C treatment and centrifugation, the pellet should not normally be red. A red pellet indicates that the trimethine product has complexed with macromolecules, causing the product concentration in the supernatant to be underestimated and introducing error. The kit contains specialized reagents and procedures that remove macromolecular interferents and help prevent this complex formation. Observe the pellet color after the final centrifugation and contact technical support if an abnormality is observed. 2) Plant samples are strongly affected by sucrose interference, whereas animal samples are strongly affected by glucose interference. Separate equations are provided for sucrose and glucose interference. For oils and fats, either equation may be used. 3) Normal absorbance range: 532 nm is the characteristic peak of the product. Keep ΔA_{532} within 0.05-1.0. If the absorbance is outside this range, adjust the sample as described below and modify the calculation parameters accordingly. A preliminary test is recommended. If ΔA_{532} is below 0.05, prepare a more concentrated sample and repeat the assay. Tissue sample concentration may be increased from 10% (w/v) to a maximum of 20% (w/v). Bacterial or cell sample concentration may be increased from 5×10^6/mL to a maximum of 2×10^7/mL. If ΔA_{532} is above 1.0, appropriately dilute the sample with distilled water and repeat the assay. 4) The 450 nm and 600 nm wavelengths correspond to characteristic peaks of sugar interferents in the sample. ΔA_{450} and ΔA_{600} are related to interferent content and should each be kept below 1.0. 5) After changing the wavelength, zero the spectrophotometer again with distilled water before measurement. 6) To ensure accurate results and avoid reagent loss, read the instructions carefully before the assay, confirm that reagent storage and

	preparation are adequate, and ensure that all operating steps are clear. Perform a preliminary test with 2-3 samples expected to show substantial differences.
Storage	Kit Components and Storage: The kit is stored at 2-8°C. Extraction Solution: liquid, 60 mL × 1 bottle; store at 4°C. Reagent 1: liquid, 8 mL × 1 bottle; store at 4°C protected from light. Reagent 2, Component A: powder, 2 bottles; store at 4°C protected from light. Reagent 2, Component B: liquid, 20 mL × 2 bottles; store at 4°C protected from light. Reagent 3: liquid, 15 mL × 1 bottle; store at 4°C. Before use, add 20 mL Component B to one bottle of Component A and dissolve completely to prepare the Detection Working Solution. The prepared Detection Working Solution can be stored at 4°C for 1 month. The Detection Working Solution is relatively difficult to dissolve; heating at 60-70°C with vigorous shaking or sonication is recommended to promote dissolution. Solid precipitation may occur during low-temperature storage and is normal. Allow the solution to return to room temperature and use sonication to promote redissolution before use.
Reconstitution	Detection Working Solution: Before use, add 20 mL Component B to one bottle of Component A. Dissolve completely. Heating at 60-70°C with vigorous shaking or sonication is recommended to facilitate dissolution. The prepared solution may be stored at 4°C for 1 month. If solids precipitate during refrigerated storage, allow the solution to return to room temperature and sonicate until dissolved before use.
Reaction conditions	Measure absorbance at 450 nm, 532 nm, and 600 nm. The assay includes a 5 min treatment at 100°C followed by a 60 min treatment at 100°C, with ice-bath cooling and centrifugation after each heating step.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA