Lipid Peroxidation (MDA) Assay Kit

(Catalog #K739-100; 100 assays; Store kit at –20°C)

I. Introduction:

Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes. Lipid peroxidation forms Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), as natural bi-products. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. BioVision's Lipid Peroxidation Assay Kit provides a convenient tool for sensitive detection of the MDA in a variety of samples. The MDA in the sample is reacted with Thiobarbituric Acid (TBA) to generate the MDA-TBA adduct. The MDA-TBA adduct can be easily quantified colorimetrically (λ = 532 nm) or fluorometrically (Ex/Em = 532/553 nm). This assay detects MDA levels as low as 1 nmol/well colorimetrically and 0.1 nmol/well fluorometrically.

II. Kit Contents:

Components	K739-100	Cap Code	Part No.
MDA Lysis Buffer	25 ml	WM	K739-100-1
Phosphotungstic Acid Solution	12.5 ml	NM	K739-100-2
BHT (100X)	1 ml	Purple	K739-100-3
TBA	4 bottles	NM	K739-100-4
MDA Standard (4.17M)	100 µl	Yellow	K739-100-5

III. Storage and Handling:

Store the kit at -20°C, protected from light. Allow all components to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay. Store all reagents at -20°C between use.

IV. Reagent Reconstitution:

Reconstitute one vial of TBA with 7.5 ml Glacial Acetic Acid (not provided), then adjust the final volume to 25 ml with ddH_2O. Store at room temperature. **Must be used within 6-8 hr.**

V. MDA Quantification Assay Protocol:

1. **Sample Preparation**: For tissue and cell samples, 10 mg tissue or cells (1×10^6) can be homogenized on ice in 300 µl of the MDA Lysis Buffer (with 3 µl BHT (100X), then centrifuged (13,000 x *g*, 10 min) to remove insoluble material. Place 200 µl of the supernatant from each homogenized sample into a microcentrifuge tube.
   For plasma samples, gently mix 10 µl plasma with 500 µl of 42 mM H_2SO_4 (not provided) in a microcentrifuge tube. Add 125 µl of Phosphotungstic Acid Solution and mix by vortexing. Incubate at room temperature for 5 min, then centrifuge for 3 min at 13,000 x *g*. Collect the pellet and resuspended on ice with 100 µl ddH_2O (with 2 µl BHT (100X)). Adjust the final volume to 200 µl with ddH_2O.
2. **MDA Standard Curve:** Dilute 10 µl of the MDA standard with 407 µl of ddH_2O to prepare a 0.1 M MDA solution, then dilute 20 µl of the 0.1 M MDA solution with 980 µl of ddH_2O to prepare a 2 mM MDA Standard. For colorimetric analysis, add 0, 2, 4, 6, 8, 10 µl of the 2 mM MDA Standard into separate microcentrifuge tubes and adjust the final volume to 200 µl with ddH_2O to generate 0, 4, 8, 12, 16 and 20 nmol Standard per well. For fluorometric analysis, dilute the 2 mM MDA Standard 10 fold (10 µl + 90 µl ddH2O), then add 0, 2, 4, 6, 8, 10 µl of the 0.2 mM MDA Standard into separate microcentrifuge tubes and adjust the final volume to 200 µl with ddH_2O to generate 0, 0.4, 0.8, 1.2, 1.6 and 2.0 nmol Standard per well.
3. **Develop:** Add 600 µl of TBA solution into each vial containing standard and sample. Incubate at 95°C for 60 min. Cool to room temperature in an ice bath for 10 min. Pipette 200 µl (from each 800 µl reaction mixture) into a 96-well microplate for analysis.
   Note: For more sensitivity, one can add 300 µl n-butanol (not provided in the kit) to extract the MDA-TBA adduct from the 800 µl reaction mixture. The layers can be separated by centrifugation (3 min at 16,000 x g). Evaporate the n-butanol and dissolve the MDA-TBA adduct in 200 µl ddH_2O then place into the 96-well microplate for analysis.
4. **Measure:** For colorimetric analysis, Read the absorbance at 532 nm.
   For the fluorometric analysis, read supernatants (Ex/Em 532/553 nm). It is recommended to set the instrument sensitivity to high with a slit width of 5 nm.
5. **Calculation:** Plot the MDA Standard Curve, then calculate the MDA amount in the test sample. Determine the sample amount of MDA equivalents in nmol by interpolation from the standard curve. Correct sample values for any other dilutions performed during specimen preparation.

C = [(A/(mg or ml)] x 4 x D = nmol/ml or nmol/mg

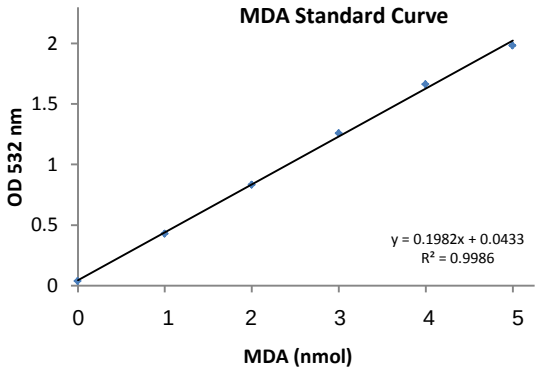
Where: **A :** Sample MDA amount from the standard curve (in nmol).

mg : Original tissue amount used (e.g. 10 mg)

ml : Original plasma volume used (e.g. 0.010 ml)

4 : Correction for using 200 µl of the 800 µl reaction mix

D : Dilution factor (if any BEFORE original amount or volume)



VI. Related Products:

NAD/NADH Quantification Kit	ADP/ATP Ratio Assay Kit
NADP/NADPH Quantification Kit	Ascorbic Acid Assay Kits
Fatty Acid Assay Kit	Glutathione Assay Kits
Uric Acid Assay Kit	GST Activity Assay Kit
Total Antioxidant Capacity (TAC) Assay Kit	SOD Activity Assay Kit
Hydrogen Peroxide Assay Kit	Triglyceride Assay Kit
Nitric Oxide Assay Kit	Glutamate Assay Kit
Catalase Activity Assay Kit	DNA Damage Quantification Kit
Thioredoxin Activty assay Kit	Proteosome Activity Assay Kit