Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit

(Catalog #BN00955; 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 biproducts. Measuring end products of lipid peroxidation is a useful measure of oxidative damage. Assay Genie's Lipid Peroxidation Assay Kit provides a convenient tool for sensitive detection of MDA in a variety of samples. MDA in the sample is reacted with Thiobarbituric Acid (TBA) to generate the MDA-TBA adduct which can be easily quantified colorimetrically (OD 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	BN00955	Cap Code	Part No.
MDA Lysis Buffer	25 ml	WM	BN00955-1
Phosphotungstic Acid Solution	12.5 ml	NM	BN00955-2
BHT (100X)	1 ml	Purple	BN00955-3
TBA	4 bottles	NM	BN00955-4
MDA Standard (4.17M)	100 µl	Yellow	BN00955-5

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. We recommend using a flat bottom, opaque, black 96-well plate for enhanced sensitivity when performing Fluorometric assays.

IV. Reagent Reconstitution:

Take one vial of TBA and add of 7.5 ml Glacial Acetic Acid (not provided) and mix. Transfer the slurry to another tube and add dH_2O to a final volume of 25 ml. Mix well to dissolve. Sonication can be used to assist dissolution if necessary. Store at 4°C.**Stable for 1 week.**

V. MDA Quantification Assay Protocol:

1. **Sample Preparation**: For tissue or cells, 10 mg (1×10^6) can be homogenized on ice in 300 µl of MDA Lysis Buffer (with 3 µl BHT (100X), then centrifuged (13,000 X *g*, 10 min.) to remove insoluble material. Alternatively, protein can be precipitated by homogenizing 10 mg sample in 150 µl dH2O + 3 µl BHT and adding 1 vol of 2N perchloric acid, vortexing, and centrifuging to remove precipitated protein. Place 200 µl of the supernatant from each sample into a microcentrifuge tube.
   For plasma samples: mix 20 µl with 500 µl of 42 mM H_2SO_4 (not provided) in a microcentrifuge tube. Add 125 µl of Phosphotungstic Acid Solution and vortex. Incubate at room temperature for 5 min., then centrifuge for 3 min. at 13,000 x *g*. Collect the pellet and resuspend on ice with 100 µl ddH_2O (with 2 µl BHT). 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 volume to 200 µl with ddH_2O to generate five standards (See Step 3). 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 volume to 200 µl with ddH_2O to generate five standards. (See step 3)
3. **Development:** Add 600 µl of TBA reagent into each vial containing standards 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 (Standard Curves Ranges. For colorimetric: 0-1-2-3-4-5 nmol MDA/well and Fluorometric: 0-0.1-0.2-0.3-0.4, 0.5 nmol MDA/well). For plasma: Mix with 300 µl of n-butanol, and 100 µl 5 M NaCl. Vortex. Centrifuge (3 min, 16,000g, RT), and keep the top layer (n-butanol). Transfer the n-butanol layer to a new centrifuge tube and remove the n-butanol (freeze-dry or oven-dry at 55 °C can be used to evaporate n-butanol). Resuspend the remaining material in 200 µl ddH_2O. Mix well and add 200 µl to a 96-well black plate.
   Occasionally, samples will exhibit a turbidity which can be eliminated by filtering through an 0.2 µm filter. TBA can react with other compounds in samples giving other colored compounds. These should not generally interfere with quantitation of the TBA-MDA adduct.
   Note: For enhanced 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. If you don't get separation, add 100 µl of 5 M NaCl and vortex vigorously. The layers can be separated by centrifugation (3 min, 16,000g, RT). Transfer and 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 and determine the MDA amount in the test sample 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)

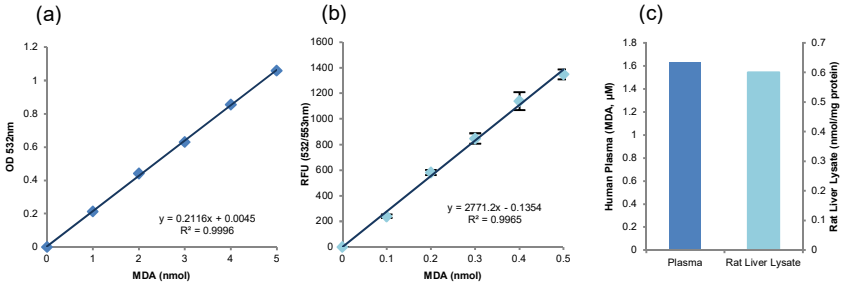

Figure: MDA Standard Curve: Colorimetric (a) and Fluorometric (b). (c) Measurement of MDA in human plasma (20 µl) and rat liver lysate (10 mg). Assay was performed following the kit protocols.

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