MA-0102

Lipid Peroxidation (MDA) Assay

(100 wells, Colorimetric, OD 532 nm / Fluorometric, Ex/Em = 532/553 nm, Store at 4°C)

Background Information:

Lipid peroxidation is a non-enzymatic oxidation process of a variety of various lipid molecules, involving abstraction of a hydrogen atom and insertion of an oxygen molecule typically at carbon atoms adjacent to a double bond. This fractures the lipid molecule resulting in the formation of products like malondialdehyde (MDA) or hydroxynonenal (HNE). These end products may act as signaling molecules. Measurement of lipid peroxidation end products is a useful measure of oxidative damage in pathophysiological processes. AkrivisBio's Lipid Peroxidation Assay provides an efficient tool for detection of MDA through reaction with Thiobarbituric Acid to generate a chromophore with absorbance at 532 nm or fluorescence (Ex/Em = 532/553 nm).

Assay Principle:

1 - Disrupt tissues or cells, precipitate protein. Use clear supernatant for analysis.
2 – Add Thiobarbituric acid, incubate at 95°C for 60 min to form chromophore.

Assay Components:

Assay Buffer	25 ml	WM	MA-0102A
Phosphotungstic Acid Solution	12.5 ml	NM	MA-0102B
BHT	1 ml	Purple	MA-0102C
Thiobarbituric Acid (TBA)	4 x 250 mg	NM	MA-0102D
MDA Standard	100 µl	Yellow	MA-0102E

User Supplied Reagents and Equipment:

Glacial Acetic Acid – 42 mM H_2SO_4 - clear flat bottom 96-well plate - Oven or hotplate – Plate reader - Eppendorf tubes or similar.

Storage and Handling:

Store the kit at 4°C. Warm everything to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay. Use a black or white plate when performing fluorescent assays.

Reagent Reconstitution:

Add of 7.5 ml Glacial Acetic Acid (user provided) to one vial of Thiobarbituric Acid and mix. Transfer the slurry to another tube and add DI H_2O to a final volume of 25 ml. Mix well to dissolve. Sonicate to assist dissolution if necessary. Store at 4°C for up to 1 week.

Assay Protocol:

1. Standard Curve: Dilute 10 µl of the MDA standard with 407 µl of DI H_2O to prepare a 0.1 M MDA solution. Dilute 20 µl of the 0.1 M MDA solution with 980 µl of DI H_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 DI H_2O. For fluorometric analysis, dilute the 2 mM MDA Standard 10-fold (10 µl + 90 µl DI H_2O). Add 0, 2, 4, 6, 8, 10 µl of the 0.2 mM MDA Standard to separate microcentrifuge tubes and adjust the volume to 200 µl with DI H_2O.

2. Sample Preparation: Homogenize 10 mg tissue or 1×10^6 cells on ice in 300 µl of MDA Lysis Buffer + 3 µl BHT. Centrifuge (13,000 X *g*, 3-5 min) to pellet insoluble material. Alternatively, precipitate protein by homogenizing the sample in 150 µl DI H_2O + 3 µl BHT. Add 150 µl 2 N perchloric acid, vortex, and centrifuge to remove protein. Transfer 200 µl of the supernatant to a microcentrifuge tube.

For plasma: Add 20 µl to 500 µl of 42 mM H_2SO_4 (user supplied) in a microcentrifuge tube. Add 125 µl of Phosphotungstic Acid Solution and vortex. Leave for 5 min, then centrifuge for 1 min. at 13,000 x *g*. Collect the pellet and resuspend with 100 µl DI H_2O + 2 µl BHT on ice. Adjust the final volume to 200 µl with DI H_2O.

3. Color Development Reaction:

Add 600 µl of TBA reagent to each vial containing standards or samples. Allow full color development to occur at 95°C for 60 min. Cool to room temperature in an ice bath for 10 min. Pipette 200 µl from each reaction mixture into a 96-well microplate for analysis. Standard Curve Ranges: Colorimetric: 0 - 5 nmol; Fluorometric: 0 - 0.5 nmol MDA. For plasma: Mix with 300 µl of n-butanol and 100 µl 5 M NaCl; Vortex; Centrifuge (3 min, 16,000g, RT); Transfer the n-butanol (top) layer to a new centrifuge tube and evaporate the n-butanol using heat (55°C) and/or vacuum. Resuspend the remaining material in 200 µl DI H_2O. Mix well and add 200 µl to a 96-well black plate.

Occasionally, samples exhibit turbidity which can be removed using a 0.22 µm filter. TBA reacts with other compounds in samples giving other colored compounds. These don't generally interfere with quantitation of the TBA-MDA adduct.

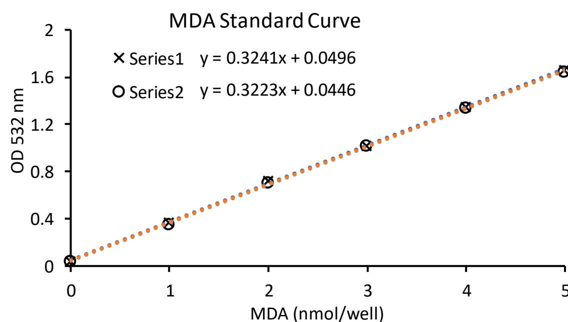
Note: For enhanced sensitivity, add 300 µl n-butanol (user provided) to extract the chromophore from the 800 µl reaction mixture. If you don't get separation, add 100 µl of 5 M NaCl and vortex vigorously. Separate the layers by centrifugation (3 min, 16,000g, RT). Transfer and evaporate the n-butanol and dissolve the chromophore in 200 µl DI H_2O then read in a 96-well microplate.

4. Measurement:

For colorimetric analysis, Read the absorbance at 532 nm.
For fluorometric analysis, read supernatants using (Ex/Em = 532/553 nm)

Typical Result:

Standard nmol Standard	Background Raw Values		Corrected Values OD	
0	0.0407	0.0420	0	0
1	0.3657	0.3536	0.3250	0.3116
2	0.7238	0.7060	0.6831	0.6640
3	1.0205	1.0160	0.9798	0.9740
4	1.3477	1.3405	1.3070	1.2985
5	1.6608	1.6439	1.6201	1.6019



5. Calculations:

Subtract the 0 MDA standard from all other Standard and Sample readings. Plot the MDA Standard Curve. Determine the slope of the Standard Curve. Divide the background corrected Sample readings by the slope of the Standard Curve to convert from OD to nanomoles in the well. To convert back to nmoles in the original sample:

A. Divide the nmoles in the well by the volume of sample added to the well (in µl) = nmoles MDA per µl of sample
B. Multiply the nmoles MDA per µl of sample X volume of supernatant/ total liquid volume in step 2 above = total nmoles MDA per sample
C. Divide total nmoles MDA per sample X mg tissue or # of cells used to prepare sample = nmoles MDA per mg sample (or per # of cells, etc.)

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