Product Information

Lipid Peroxidation (MDA) Assay Kit

Catalog Number MAK085
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Lipid peroxidation is the degradation of lipids that occurs as a result of oxidative damage and is a useful marker for oxidative stress. Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction with the production of end products such as malondialdehyde (MDA). Lipid peroxidation may contribute to the pathology of many diseases including atherosclerosis, diabetes, and Alzheimer’s.

In this kit, lipid peroxidation is determined by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm)/fluorometric (\(\lambda_{\text{ex}} = 532\), \(\lambda_{\text{em}} = 553\) nm) product, proportional to the MDA present.

Components

The kit is sufficient for 100 assays in 96 well plates.

MDA Lysis Buffer
Catalog Number MAK085A
25 mL

Phosphotungstic Acid Solution
Catalog Number MAK085B
12.5 mL

BHT, 100×
Catalog Number MAK085C
1 mL

TBA
Catalog Number MAK085D
4 bottles

MDA Standard, 4.17 M
Catalog Number MAK085E
0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader.
- Glacial acetic acid (Catalog Number A6283 or equivalent)
- Perchloric acid (Catalog Number 244252 or equivalent)
- Sulfuric acid (Catalog Number 258105 or equivalent)
- 1-Butanol (Catalog Number 360465 or equivalent)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Use ultrapure water for the preparation of all reagents. Allow all components to come to room temperature before starting.

TBA Solution – Reconstitute a bottle with 7.5 mL Glacial Acetic Acid, then adjust the final volume to 25 mL with water. Sonication can be used to assist dissolution if necessary. Store at 4 ºC and use within 1 week of preparation.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 ºC, protected from light, is recommended.
**Procedure**

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of all standards and samples.

**MDA Standards for Colorimetric Detection**

Dilute 10 μL of the 4.17 M MDA Standard Solution with 407 μL of water to prepare a 0.1 M MDA Standard Solution. Further dilute 20 μL of the 0.1 M MDA Standard Solution with 980 μL of water to prepare a 2 mM MDA Standard. Add 0, 2, 4, 6, 8, and 10 μL of the 2 mM MDA Standard Solution into separate microcentrifuge tubes, generating 0 (blank), 4, 8, 12, 16, and 20 nmole standards. Add water to each tube to bring the volume to 200 μL.

**MDA Standards Fluorometric Detection**

Prepare a 2 mM Standard Solution as for the colorimetric assay. Take 100 μL of the 2 mM MDA Standard Solution and add to 900 μL of water to make a 0.2 mM MDA standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 0.2 mM MDA standard solution into separate microcentrifuge tubes, generating 0 (blank), 0.4, 0.8, 1.2, 1.6, and 2.0 nmole standards. Add water to each tube to bring the volume to 200 μL.

**Sample Preparation**

Serum or Plasma samples (20 μL) should be gently mixed with 500 μL of 42 mM sulfuric acid in a microcentrifuge tube. Add 125 μL of Phosphotungstic Acid Solution and mix by vortexing. Incubate at room temperature for 5 minutes and then centrifuge the samples at 13,000 × g for 3 minutes. In a separate tube, add 2 μL of BHT (100×) to 100 μL of water. Resuspend the pellet on ice with the water/BHT solution. Adjust the volume to 200 μL with water.

Tissue (10 mg) or cells (1 × 10⁵) can be homogenized on ice in 300 μL of the MDA Lysis Buffer containing 3 μL of BHT (100×). Centrifuge the samples at 13,000 × g for 10 minutes to remove insoluble material. Alternatively, protein can be precipitated by homogenizing 10 mg of sample in 150 μL of water containing 3 μL of BHT (100×) and adding 1 volume of 2 N perchloric acid, vortexing, and centrifuging to remove precipitated protein. Place 200 μL of the supernatant from each homogenized sample into a microcentrifuge tube.

**Assay Reaction**

1. To form the MDA-TBA adduct, add 600 μL of the TBA solution into each vial containing standard and sample. Incubate at 95 ºC for 60 minutes. Cool to room temperature in an ice bath for 10 minutes.

2. Pipette 200 μL from each reaction mixture, except for Serum or Plasma samples (see step 3), into a 96 well plate for analysis.

   Notes: To enhance sensitivity, one can add 300 μL of 1-butanol to extract the MDA-TBA adduct from the 800 μL reaction mixture. If separation does not occur, add 100 μL of 5 M NaCl and vortex vigorously. Centrifuge at 16,000 × g for 3 minutes at room temperature to separate the layers. Transfer the 1-butanol layer (the top layer) to another tube, and evaporate the 1-butanol. The 1-butanol can be removed either by freeze-drying, or heating on a hot block at 55 ºC. Dissolve the residue containing the MDA-TBA adduct in 200 μL of water, and then transfer to a 96 well plate for analysis.

   Occasionally samples will exhibit turbidity, which can be eliminated by filtering through a 0.2 μm filter.

   TBA can react with other compounds in samples giving other colored products. These should not generally interfere with quantitation of the TBA-MDA adduct.

3. For Serum or Plasma samples, mix with 300 μL of 1-butanol and 100 μL of 5 M NaCl with each reaction mixture. Vortex and then centrifuge for 3 minutes at 16,000 × g at room temperature. Transfer the 1-butanol layer (the top layer) to a new centrifuge tube and remove the 1-butanol. The 1-butanol can be removed either by freeze-drying, or heating on a hot block at 55 ºC. Resuspend the remaining material in 200 μL of ultrapure water. Mix well and add 200 μL into a 96 well plate.

4. For colorimetric assays, measure the absorbance at 532 nm (A₅₃₂). For fluorometric assays, measure fluorescence intensity (λₑₓ = 532/λₑᵐ = 553 nm).
Results
Calculations
The background for either assay is the value obtained for the 0 (blank) MDA standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate MDA standards to plot a standard curve. The amount of MDA present in the samples may be determined from the standard curve. Note: A new standard curve must be set up each time the assay is run.

Concentration of MDA for samples without 1-butanol concentration step

\[(S_a/S_v) \times D = C\]

\(S_a = \) Amount of MDA in unknown sample (nmole) from standard curve
\(S_v = \) Sample volume (µL) or amount (mg) added into the wells
\(C = \) Concentration of MDA in sample
\(D = \) Sample dilution factor (if applicable)

Sample Calculation
Amount of MDA \((S_a) = 5.84\) nmole
Sample volume \((S_v) = 20\) µL

Concentration of MDA in sample

\[(5.84 \text{ nmole}/20 \text{ µL}) \times 1 = 0.292 \text{ nmole}/µL\]

Concentration of MDA for samples with 1-butanol concentration step

\[(S_a/S_v) \times 4 \times D = C\]

\(S_a = \) Amount of MDA in unknown sample (nmole) from standard curve
\(S_v = \) Sample volume (µL) or amount (mg) added into the wells
\(C = \) Concentration of MDA in sample
\(D = \) Sample dilution factor (if applicable)
\(4 = \) Correction factor for using 200 µL of the 800 µL reaction

Sample Calculation
Amount of MDA \((S_a) = 5.84\) nmole
Sample volume \((S_v) = 20\) µL

Concentration of MDA in sample

\[(5.84 \text{ nmole}/20 \text{ µL}) \times 4 \times 1 = 1.168 \text{ nmole}/µL\]
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Cold assay buffer</td>
<td>Assay Buffer must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
</tr>
<tr>
<td></td>
<td>Plate reader at incorrect wavelength</td>
<td>Check filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Type of 96 well plate used</td>
<td>For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates</td>
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<tr>
<td>Samples with erratic readings</td>
<td>Samples prepared in different buffer</td>
<td>Use the Assay Buffer provided or refer to Technical Bulletin for instructions</td>
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<tr>
<td></td>
<td>Cell/Tissue culture samples were incompletely homogenized</td>
<td>Repeat the sample homogenization, increasing the length and extent of homogenization step.</td>
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<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if samples will be used multiple times</td>
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<td></td>
<td>Presence of interfering substance in the sample</td>
<td>If possible, dilute sample further</td>
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<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples and store correctly until use</td>
</tr>
<tr>
<td>Lower/higher readings in samples and standards</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
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<td>Use of expired kit or improperly stored reagents</td>
<td>Check the expiration date and store the components appropriately</td>
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<td></td>
<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh reaction mix before use</td>
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<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
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<td></td>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
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<tr>
<td>Non-linear standard curve</td>
<td>Use of partially thawed components</td>
<td>Thaw and resuspend all components before preparing the reaction mix</td>
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<td></td>
<td>Pipetting errors in preparation of standards</td>
<td>Avoid pipetting small volumes</td>
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<td></td>
<td>Pipetting errors in the Reaction Mix</td>
<td>Prepare a master Reaction Mix whenever possible</td>
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<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the plate well</td>
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<td>Standard stock is at incorrect concentration</td>
<td>Refer to the standard dilution instructions in the Technical Bulletin</td>
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<tr>
<td></td>
<td>Calculation errors</td>
<td>Recalculate calculations after referring to Technical Bulletin</td>
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<tr>
<td></td>
<td>Substituting reagents from older kits/lots</td>
<td>Use fresh components from the same kit</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
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