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 (λ<sub>exc</sub> = 532/λ<sub>em</sub> = 553 nm) product, proportional to the MDA present.

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

- MDA Lysis Buffer 25 mL
  Catalog Number MAK085A
- Phosphotungstic Acid Solution 12.5 mL
  Catalog Number MAK085B
- BHT, 100× 1 mL
  Catalog Number MAK085C
- TBA 4 bottles
  Catalog Number MAK085D
- MDA Standard, 4.17 M 0.1 mL
  Catalog Number MAK085E

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 room temperature and use within 1 week of preparation.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, 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 (10 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 × 10⁶) 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 °C for 60 minutes. Cool to room temperature in an ice bath for 10 minutes. Pipette 200 µL from each reaction mixture into a 96 well plate for analysis.
   Notes: 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.

   To enhance sensitivity, 300 µL of 1-butanol can be added to 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 to separate the layers. Transfer the 1-butanol, dissolve the MDA-TBA adduct in 200 µL of water, and then transfer to a 96 well plate for analysis.

2. 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 (\(\mu\)L) or amount (\(\mu\)g) 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 (\(S_v\)) = 50 \(\mu\)L

Concentration of MDA in sample

\[(5.84 \text{ nmole}/50 \text{ \(\mu\)L}) \times 1 = 0.1168 \text{ nmole/\(\mu\)L}\]

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 (\(\mu\)L) or amount (\(\mu\)g) added into the wells
\(C\) = Concentration of MDA in sample
\(D\) = Sample dilution factor (if applicable)
4 = Correction factor for concentrating 800 \(\mu\)L reaction into 200 \(\mu\)L

Sample Calculation
Amount of MDA (\(S_a\)) = 5.84 nmole
Sample volume (\(S_v\)) = 50 \(\mu\)L

Concentration of MDA in sample

\[(5.84 \text{ nmole}/50 \text{ \(\mu\)L}) \times 4 \times 1 = 0.4672 \text{ nmole/\(\mu\)L}\]
### Troubleshooting Guide

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<tr>
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<td>For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates</td>
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<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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<td>Repeat the sample homogenization, increasing the length and extent of homogenization step.</td>
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<td>Aliquot and freeze samples if samples will be used multiple times</td>
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<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>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh reaction mix before use</td>
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<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
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<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
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<td>Avoid pipetting small volumes</td>
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<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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<td>Samples measured at incorrect wavelength</td>
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<td>Samples contain interfering substances</td>
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<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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