Technical Bulletin

Lipid Peroxidation (MDA) Assay Kit

Catalog Number MAK085

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_{Ex} = 532/λ_{Em} = 553 nm) product, proportional to the MDA present.

Components

The kit is sufficient for 100 colorimetric or fluorometric 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

- Pipetting devices and accessories (e.g., multichannel pipettor)
- 96-well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- 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

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.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Use purified 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 of 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 °C and use within 1 week of preparation.

Procedure

All samples and standards should be run in duplicate.

Use purified water for the preparation of all standards and samples.

MDA Standards for Colorimetric Detection

1. A new standard curve must be set up each time the assay is run.
2. Dilute 10 µL of the 4.17 M MDA Standard Solution with 407 µL of purified water to prepare a 0.1 M MDA Standard Solution.
3. Further dilute 20 µL of the 0.1 M MDA Standard Solution with 980 µL of purified water to prepare a 2 mM MDA Standard.
4. Prepare MDA standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of MDA Standards for Colorimetric Assay

Well	2 mM MDA Standard	Purified Water	MDA (nmole/well)
1	10 µL	190 µL	20
2	8 µL	192 µL	16
3	6 µL	194 µL	12
4	4 µL	196 µL	8
5	2 µL	198 µL	4
6	-	200 µL	0

MDA Standards for Fluorometric Detection

1. A new standard curve must be set up each time the assay is run.
2. Prepare a 2 mM Standard Solution as for the Colorimetric assay Steps 1 and 2.
3. Dilute 100 µL of the 2 mM MDA Standard Solution with 900 µL of water to make a 0.2 mM MDA standard solution.
4. Prepare MDA standards in 1.5 mL microcentrifuge tubes according to Table 2.

Table 2.
Preparation of MDA Standards for Fluorometric Assay

Well	0.2 mM MDA Standard	Purified Water	MDA (nmole/well)
1	10 µL	190 µL	2.0
2	8 µL	192 µL	1.6
3	6 µL	194 µL	1.2
4	4 µL	196 µL	0.8
5	2 µL	198 µL	0.4
6	-	200 µL	0

Sample Preparation

Serum or Plasma

1. Gently mix 20 µL of serum or plasma samples with 500 µL of 42 mM sulfuric acid in a microcentrifuge tube.
2. Add 125 µL of Phosphotungstic Acid Solution and mix by vortexing.
3. Incubate at room temperature for 5 minutes and then centrifuge the samples at 13,000 × *g* for 3 minutes. Remove supernatant and retain pellet for assay.
4. In a separate tube, add 2 µL of BHT (100×) to 100 µL of purified water.
5. Resuspend the pellet on ice with the water/BHT solution. Adjust the volume to 200 µL with water.

Tissue or Cells

1. Homogenize tissue (10 mg) or cells (2×10^6) on ice in 300 µL of the MDA Lysis Buffer containing 3 µL of BHT (100×).
2. 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 purified water containing 3 µL of BHT (100×) and adding 1 volume of 2 N perchloric acid, vortexing, and centrifuging to remove precipitated protein.

3. 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.
2. Incubate at 95 °C for 60 minutes.
3. Cool to room temperature in an ice bath for 10 minutes.
4. Pipette 200 µL from each Standard and Sample reaction mixture, except for Serum or Plasma Samples (see Step 5), into a 96-well plate for analysis.

   Note:

   a. To enhance sensitivity, 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 °C. Dissolve the residue containing the MDA-TBA adduct in 200 µL of purified water, and then transfer to a 96-well plate for analysis.
   b. Occasionally samples will exhibit turbidity, which can be eliminated by filtering through a 0.2 µm filter.
   c. TBA can react with other compounds in Samples to produce other colored products. These should not generally interfere with quantitation of the TBA-MDA adduct.

5. For Serum or Plasma Samples, mix each reaction mixture with 300 µL of 1-butanol and 100 µL of 5 M NaCl. 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 °C. Resuspend the remaining material in 200 µL of ultrapure water. Mix well and add 200 µL into a 96-well plate.

Measurement

For colorimetric assays, measure the absorbance (A) at 532 nm. For fluorometric assays, measure relative fluorescence units (RFU) at λ_{Ex} = 532 nm /λ_{Em} = 553 nm).

Results

1. Calculate ΔA / ΔRFU by subtracting the A / RFU reading of Standard #6 (Blank) from the remaining Standard reading values. Background values can be significant and must be subtracted from all readings. The amount of MDA present in the samples may be determined from the standard curve.
2. Plot the ΔA / ΔRFU values against Standard concentrations and determine the slope of the standard curve.
3. Using the standard curve determine the quantity of MDA in nmole in the Sample.

   a. For Samples without the 1-butanol concentration step, calculate the MDA concentration of the Sample:

   MDA (nmol/mL) =

$$(S_A/S_V) \times DF = C$$

where:

S_A = Amount of MDA in Sample (nmole) as determined from the standard curve

S_V = Sample volume (mL) or amount (mg) added into the wells

DF = Sample dilution factor (DF = 1 for undiluted Samples)

C = Concentration of MDA in sample

Example Calculation

Amount of MDA (S_A) = 5.84 nmole

Sample volume (S_V) = 0.020 mL

Concentration of MDA in sample =

(5.84 nmole/0.020 mL) × 1 = 292 nmole/mL

b. For Samples with the 1-butanol concentration step, calculate the MDA concentration of the Sample:

MDA (nmol/mL) =

$$(S_A/S_V) \times 4 \times DF = C$$

where:

S_A = Amount of MDA in Sample (nmole) as determined from the standard curve

S_V = Sample volume (mL) or amount (mg) added into the wells

4 = Correction factor for using 200 µL of the 800 µL reaction

DF = Sample dilution factor (DF = 1 for undiluted Samples)

C = Concentration of MDA in sample

Example Calculation

Amount of MDA (S_A) = 5.84 nmole

Sample volume (S_V) = 0.020 mL

Concentration of MDA in sample

(5.84 nmole/0.020 mL) × 4 × 1 = 1,168 nmole/mL

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