Technical Bulletin

Lipid Peroxidation (MDA) Assay Kit

Catalogue number MAK568

Product Description

Lipid peroxidation (LP) is the degradation of lipids that occurs as a result of oxidative damage and is a useful marker for oxidative stress in biological systems. One of the most common ways to assess LP relies on the detection and quantification of malondialdehyde (MDA), which is a common product of peroxidation of polyunsaturated fatty acids[1]. MDA and other LP-related molecules can indicate oxidative stress by reacting with thiobarbituric acid (TBA) to form products which are called thiobarbituric acid reactive substances (TBARS). These are molecules that can be detected by various spectrometric methods.

The Lipid Peroxidation Assay Kit measures the concentration of MDA in various biological samples. The method is based on the detection and quantification of the adduct formed by the reaction of MDA and TBA in acidic conditions. The resulting product can be detected colorimetrically (absorption = 532 nm) or fluorometrically ($\lambda_{Ex}/\lambda_{Em}$ = 530/560 nm).

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Lysis Buffer — 25 mL
  Catalogue Number MAK568A
- Phosphotungstic Acid Solution — 12.5 mL
  Catalogue Number MAK568B
- BHT Stock/BHT (X100) — 1 mL
  Catalogue Number MAK568C
- TBA — 4 x 0.25 g
  Catalogue Number MAK568D
- MDA Standard — 0.1 mL
  Catalogue Number MAK568E

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plate.
  - Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent)
  - Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)
  - Cell culture or tissue culture treated plates are not recommended.
- Plate reader that is capable to read absorbance at 532 nm or fluorescence at $\lambda_{Ex}/\lambda_{Em}$ 530/560 nm.
- Pipettors and Pipettes
- Vortex Mixer
- Sonicator
- Glacial acetic acid (Catalogue Number A6283 or equivalent)
- Perchloric acid (Catalogue Number 244252 or equivalent)
- Sulfuric acid (Catalogue Number 258105 or equivalent)
- 1-Butanol

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 product is shipped on dry ice. Store at -20°C upon receipt.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Avoid repeated freeze/thaw cycles.

Preparation of TBA Solution

1. Transfer the contents of 1 TBA vial (MAK568D) into a 50 mL tube and add 7.5 mL glacial acetic acid (if there are TBA residues in the empty vial, wash it carefully using 7.5 mL of acetic acid).
2. Adjust the final volume to 25 mL with ultrapure water and vortex well until all solids are dissolved. If the material does not dissolve, sonicate for several minutes.
3. Store at 4°C and use within 1 week.

Procedure

All samples and standards should be run in technical duplicates or triplicates.

Sample Preparation

Serum or Plasma*:

1. Mix 20 µL serum or plasma with 500 µL of 42 mM sulfuric acid in a microcentrifuge tube.
2. Add 125 µL Phosphotungstic acid solution and mix by vortexing.
3. Incubate at room temperature for 5 minutes and then centrifuge the samples at 13,000 x g for 3 minutes. Discard supernatant and retain pellet for the assay.
4. Add 2 µL BHT Solution to 100 µL cold ultrapure water and then add to pellet. Resuspend by vortex or sonication. Bring final volume to 200 µL with water.

* It is recommended to perform butanol extraction, as described in note 3.

Notes:

1. Samples that exhibit pronounced turbidity may be filtered through a 0.2 µm filter.
2. TBA can react with other compounds in organic samples to produce other colored products. These generally should not interfere with the quantitation of the TBA-MDA adduct.
3. To enhance sensitivity or to detect MDA in samples with low concentrations (such as plasma/serum/urine), it is advised to perform butanol extraction, as follows:

   3.1 After the 1-hour reaction with TBA, cool the sample to room temperature.

   3.2 Add 300 µL 1-butanol and 100 µL 5M NaCl to each vial. Vortex and then centrifuge at 16,000 x g for 10 minutes. Collect the top layer of ~ 300 µL and transfer to a new vial.

   3.3 Evaporate the butanol by freeze-drying or by incubating at 55°C overnight.

   3.4 Dissolve the TBA-MDA adduct in 200 µL water, transfer to a 96-well plate and perform reading.

Tissues 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.
2. Centrifuge the samples at 13,000 x g for 10 minutes to remove insoluble material. Collect supernatant.

For samples containing high amounts of protein, the following procedure is recommended:

1. Homogenize tissue (10 mg) on ice in 150 µL water + 3 µL BHT.
2. Add 150 µL 2N perchloric acid and vortex.
3. Centrifuge the samples at 13,000 x g for 10 minutes. Collect supernatant.

Preparation of MDA Standards

A new standard curve should be prepared for every assay.

1. Dilute 10 µL of the MDA standard in 595 uL water to create a 0.1 M MDA solution. Mix by vortexing and ensure there are no micelles or turbidity.
2. Further dilute 20 µL of the 0.1 M MDA solution in 980 µL water to create a 2 mM MDA standard solution.
3. Prepare the colorimetric MDA standards in suitable microcentrifuge tubes according to Table 1.

Preparation of Colorimetric MDA Standards.

Tube	2 mM MDA Standard (µL)	Water (µL)	Concentration nmol/well (µM)
1	0	600	0
2	6	594	4 (20)
3	12	588	8 (40)
4	18	582	12 (60)
5	24	576	16 (80)
6	30	570	20 (100)

4. For the fluorometric assay, further dilute 20 µL of the 2 mM MDA solution in 980 µL water to create a 0.2 mM MDA standard solution.
5. Prepare the fluorometric MDA standards in suitable microcentrifuge tubes according to Table 2

Table 2.

Preparation of Fluorometric MDA Standards.

Tube	0.2 mM MDA Standard (µL)	Water (µL)	Concentration nmol/well (µM)
1	0	600	0
2	6	594	0.4 (2)
3	12	588	0.8 (4)
4	18	582	1.2 (6)
5	24	576	1.6 (8)
6	30	570	2.0 (10)

Assay Reaction

1. In an appropriate size tube add 600 µL TBA solution to 200 µL of sample or standard.
2. Incubate at 95°C for 1 hour.
3. Cool to room temperature in an ice bath or fridge.
4. Pipette 200 µL from each sample or standard into separate wells of a 96-well plate (clear for colorimetric and black for fluorescent assay).
5. Read the absorbance at 532 nm or fluorescence at $\lambda_{Ex}/\lambda_{Em}$ = 530/560 nm (for fluorescence reading it is advised to use slit width of 5 nm).

Results

Calculations

1. Calculate the average value for each duplicate of the standards and samples.
2. Subtract the blank (0) from all averages.
3. Plot the adjusted values of the standard points as a function of MDA concentration.
4. Determine the slope of the standard curve (this can be done by the plate reader software or by adding a trend line in a spreadsheet software like Excel).
5. Using the slope of the curve, calculate the quantity of MDA in the Sample:

For samples without the butanol extraction step:

$$\text{MDA (nmol/mL)} = (S_A/S_V) \times DF$$

For samples <u>with</u> the butanol extraction step:

$$\text{MDA (nmol/mL)} = (S_A/S_V) \times DF \times 4$$

Where:

S_A = Amount of MDA in Sample (nmol) 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)

Example Calculation (no butanol extraction)

MDA (nmol/mL) = (S_A/S_V) × DF

Amount of MDA as determined from calibration curve (S_A) = 4.66 nmol

Sample volume (S_V) = 0.2 mL

Dilution performed (DF) = 2

MDA concentration =

(4.66 nmol / 0.2 mL) × 2 = **46.6 nmol/mL**

Figure 1.

Exemplary Colorimetric Standard Curve

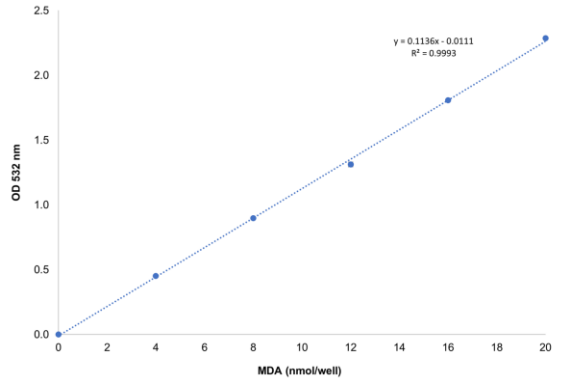


Figure 2.

Exemplary Fluorometric Standard Curve

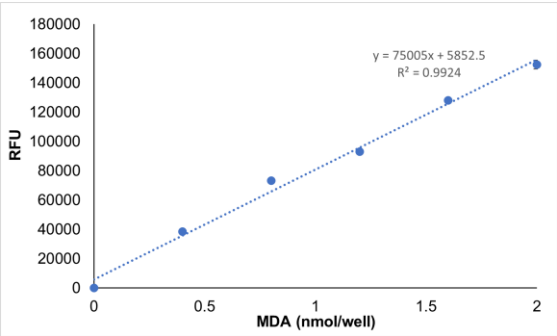


Figure 3.

Colorimetric measurement of MDA in HepG2 cells (hepatocellular carcinoma). Cells were incubated with a polyunsaturated fatty acid (PUFA – linoleic acid) that facilitates oxygen species (ROS) propagation, thus promoting lipid peroxidation. It can be seen that as PUFA concentration increases, more MDA is detected compared to control cells.

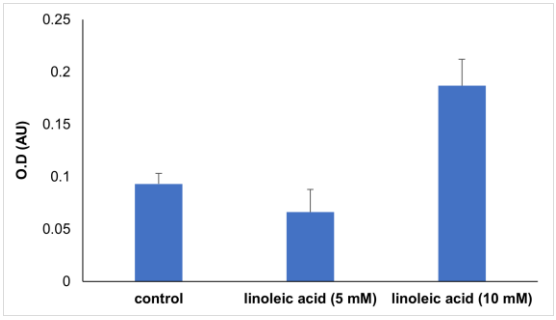
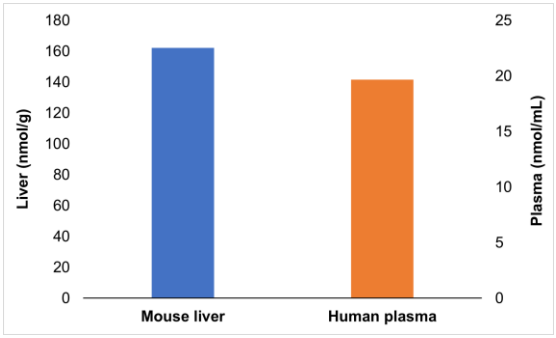


Figure 4.

Fluorometric measurement of MDA in biological samples. Mouse liver was first homogenized in ultrapure water with BHT over ice. 2N perchloric acid was added (1:1) and the mixture centrifuged. Supernatant was reacted with TBA for 1 hour and the fluorescence read. MDA in human plasma was evaluated by first precipitation with phosphotungstic acid and diluted sulfuric acid. Pellet was resuspended in DDW and BHT and reacted with TBA for 1 hour, after which the fluorescent product was extracted with butanol.



References

1. Draper, H. H. & Hadley M., *Meth. Enzymol.*, **186**, 421 (1990).

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