Lipid Peroxidation (MDA) Assay Kit

Lipid Peroxidation (MDA) Assay Kit is a detection kit for the quantification of MDA in serum, plasma, urine, cell lysate and tissue lysate by the TBARS assay.

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INTRODUCTION

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges (-CH2-) that possess especially reactive hydrogen atoms. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination. The chemical products of this oxidation are known as lipid peroxides or lipid oxidation products (LOPs).

The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). MDA appears to be the most mutagenic product of lipid peroxidation, whereas 4-HNE is the most toxic. Malondialdehyde (MDA) is the organic compound with the nominal formula CH2(CHO)2. A colorless liquid, malondialdehyde is a highly reactive compound that occurs as the enol. It occurs naturally and is a marker for oxidative stress. Malondialdehyde and other thiobarbituric reactive substances (TBARS) condense with two equivalents of thiobarbituric acid to give a fluorescent red derivative that can be assayed spectrophotometrically.

[Provide from Wikipedia]

PRINCIPLE OF THE ASSAY

Lipid Peroxidation (MDA) Assay Kit provide a simple, reproducible, and consistent system for the detection of lipid peroxidation through direct quantitative measurement of MDA in serum, plasma, urine, cell lysate and tissue lysate by TBARS assay. The unknown MDA containing samples or MDA standards are reacted with TBA to form a MDA-TBA Adduct when incubating at 95°C. The MDA-TBA adduct formed from the reaction of MDA in samples with TBA can be measured colorimetrically at a wavelength of 532 nm or fluorometrically at λ ex/em = 540/590 nm. TBARS levels are determined from a Malondialdehyde equivalence standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
SDS Lysis Solution	20 ml (Ready-to-use)	4°C
MDA Standard (1.0 mM of Malondialdehyde bis (dimethyl acetal))	1 ml	4°C
Thiobarbituric Acid (TBA)	1 g	4°C
2X TBA Acid Diluent	25 ml	4°C
Sodium Hydroxide Solution	5 ml (Ready-to-use)	4°C
100X BHT Solution	1 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 532 nm (530-540 nm) (or Fluorometric microplate reader capable of reading at 540nm excitation and 590nm emission)
- 1X PBS
- 96 well ELISA strips or 96 well microtiter plate or 96 well black fluorescence microplate for reading samples/standards
- n-Butanol
- Heat block, incubator or water bath
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the control and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Note: ***MDA adducts are not stable at long term. For best results test all samples immediately upon collection, or aliquot and store them at -80°C for up to one month. Avoid repeated freeze-thaw cycles.***

1. Tissue:
    a. Because hemoglobin interferes with the assay, blood should be removed from tissue sample by perfusion with PBS containing heparin.
    b. Weight tissue sample and resuspend tissue in PBS at concentration of 50 to 100 mg/mL.
    c. To prevent further oxidation, add 100X BHT Solution to achieve a final concentration of 1X BHT (for example, add 10 µL of 100X BHT to 1 mL of sample volume).
    d. Homogenize the tissue sample on ice. Then centrifuge at 10,000 g for 5 min at 4°C.
    e. Collect the supernatant. The supernatant can be assayed directly for its TBARS level and results can be normalized based on its protein concentration.

2. Plasma:
    a. To minimize the hemoglobin interference, prepare the plasma sample as soon as possible after blood being collected.
    b. Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 10-15 minutes at 1000 x g at 2-8°C. Collect the supernatant.
    c. To prevent further oxidation, add 100X BHT to plasma samples to achieve a final concentration of 1X (for example, add 10 µL of 100X BHT to 1 mL of plasma).
    d. Plasma samples can be assayed directly without further processing.
3. Serum:
    a. Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect the supernatant.
    b. To prevent further oxidation, add 100X BHT to Serum samples to achieve a final concentration of 1X (for example, add 10 µL of 100X BHT to 1 mL of Serum).
    c. Serum samples can be assayed directly without further processing.
4. Cells:
    a. Wash the cells once with PBS.
    b. Resuspend cells at 1-2 x 10^7 cells/mL in PBS.
    c. To prevent further oxidation, add 100X BHT Solution to achieve a final concentration of 1X BHT (for example, add 10 µL of 100X BHT to 1 mL of cell suspension).
    d. Homogenize or sonicate the cells on ice. Use the whole homogenate in the assay.

5. Urine:

   To remove insoluble particles, spin at 10,000 g for 5 min. The supernatant can be assayed directly.

REAGENT PREPARATION

- 1X TBA Acid Diluent: Dilute the 2X TBA Acid Diluent with equal parts distilled or deionized water.
- SDS Lysis Solution: If precipitated crystals are present, briefly heat the solution at 37°C to re-dissolve the SDS crystals.
- TBA Reagent: Prepare the TBA Reagent just before use. Prepare a 5.2 mg/mL solution of TBA Reagent by weighing out an amount of TBA needed for all samples and standards (eg: 130 mg of TBA is enough to prepare 100 tests). Add 1X TBA Acid Diluent to the TBA and stir or mix vigorously until the powder has dissolved (eg: 25 mL 1X TBA Diluent for 130 mg of TBA). Adjust the pH of the solution to pH 3.5 with the Sodium Hydroxide Solution.

  Note: The TBA Reagent is stable for 24 hours. Do not store or reuse diluted solutions.
- Standards: The concentration of MDA standard stock of 1 mM. The distilled water serves as zero standard (0 µg/ml), and the rest of the standard serial dilution can be diluted with distilled water as according to the suggested concentration below: 125 µM, 62.5 µM, 31.25 µM, 15.625 µM, 7.81 µM, 3.91 µM, 19.5 µM and 0.98 µM.

Dilute MDA standard as according to the table below:

Standard	Standard Conc. (µM)	µl of distilled water	µl of standard
S8	125	875	125 of 1 mM Stock
S7	62.5	250	250 of S8
S6	31.25	250	250 of S7
S5	15.625	250	250 of S6
S4	7.81	250	250 of S5
S3	3.91	250	250 of S4
S2	1.95	250	250 of S3
S1	0.98	250	250 of S2
S0	0	250	0

ASSAY PROCEDURE

Prepare and mix all reagents thoroughly before use. Samples, standards should be assayed in duplicates. High content MDA samples can be further diluted for analysis.

1. Add **100 µL** of **unknown samples and each MDA standard** to separate microcentrifuge tubes.
   Note: If needed, unknown samples may be diluted in distilled water.
2. Add **100 µL** of the **SDS Lysis Solution** to both the unknown samples and the MDA standards. Mix thoroughly. Incubate samples for **5 minutes at room temperature.**
3. Add **250 µL** of **TBA Reagent** to each sample and standard to be tested.
4. Close each tube with cap holder and incubate at **95°C for 45-60 minutes**.
5. Immediately remove tubes and cool tubes to room temperature **in an ice bath for 5 minutes.**
6. Centrifuge all sample tubes at **3000 rpm for 15 minutes at 4°C**. Collect the

supernatant from samples for further analysis.

7. **(optional)** Butanol Extraction: To prevent the interference of hemoglobin and its derivatives, we recommend the following extraction procedure:
   a. Transfer **300 µL** of the **supernatant (from step 6)** to another new tube, add **300 µL of n-Butanol**.
   b. Vortex vigorously for 1-2 minutes and **centrifuge for 5 minutes at 10,000 g at 4°C.**
   c. Transfer the butanol fraction for further measurement.
8. **For Spectrophotometric Measurement:** Transfer **200 µL** of the MDA standards and samples to a 96 well microplate compatible with a spectrophotometric plate reader. Remember to include a 0 µM blank control. It is recommended that duplicates of each standard and sample should be read. **Read the absorbance at 532nm (530-540 nm).**
9. **For Fluorometric Measurement:** Transfer **150 µL** of the MDA standards and samples to a 96 well black fluorescence microplate compatible with a fluorometric plate reader. Remember to include a 0 µM blank control. It is recommended that duplicates of each standard and sample should be read. **Read the plate at 540 nm excitation and 590 nm emission.**

CALCULATION OF RESULTS

1. Subtract the mean absorbance value of the blank (S0, Standard #0) from all standard and sample readings. This is the corrected absorbance. Calculate the average absorbance values for each set of standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean corrected absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. If the samples have been further diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF RESULTS

1. The following data is for demonstration only and cannot be used in place of data generations at the time of assay.