Product Manual

OxiSelect™ TBARS Assay Kit (MDA Quantitation)

Catalog Number

STA-330	200 assays
STA-330-5	5 x 200 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lipid peroxides are unstable indicators of oxidative stress in cells that decompose to form more complex and reactive compounds such as Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), natural bi-products of lipid peroxidation. Oxidative modification of lipids can be induced *in vitro* by a wide array of pro-oxidant agents and occurs *in vivo* during aging and in certain disease conditions. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage.

Thiobarbituric Acid Reactive Substances (TBARS) is a well-established assay for screening and monitoring lipid peroxidation. The rapid and easy protocol has been modified by researchers in the evaluation of drugs, food, as well as human and animal tissue samples. MDA forms a 1:2 adduct with thiobarbituric acid (Figure 1). The MDA-TBA adduct formed from the reaction of MDA in samples with TBA can be measured colorimetrically or fluorometrically. TBARS levels are determined from a Malondialdehyde equivalence standard.

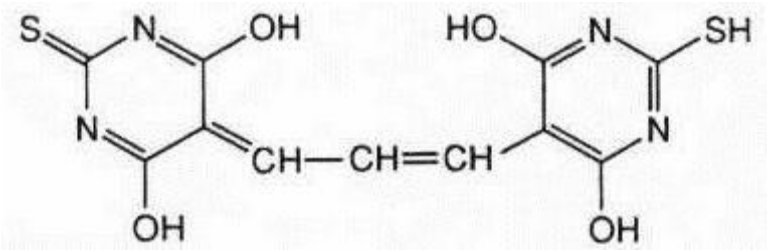

Figure 1. MDA-TBA Adduct

The TBARS Assay has provided relevant information concerning free radical activity in disease states and measurement of many compounds anti-oxidant characteristics. Although the specificity of TBARS toward compounds other than MDA has been controversial, the assay continues to be the most widely employed format for monitoring lipid peroxidation. Lipids with higher degrees of unsaturated bonds produce higher TBARS values. Interfering soluble TBARS can be minimized if lipoprotein fractions are first acid precipitated from samples. Biological samples may contain a mixture of thiobarbituric acid reactive substances such as hydroperoxides and aldehydes, which increase in response to oxidative stress. If excessively high TBARS values are obtained, a more specific assay such as HPLC should be employed.

The OxiSelect™ TBARS Assay Kit offers a simple, reproducible, and consistent system for the detection of lipid peroxidation in urine, plasma, serum, lysates, and tissue homogenates. This kit includes an MDA standard for use as a positive control. Each kit provides sufficient reagents to perform 200 tests including standard curve and unknown samples.

Assay Principle

The Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit is a tool for the direct quantitative measurement of MDA in biological samples. The unknown MDA containing samples or MDA standards are first reacted with TBA at 95°C. After a brief incubation, the samples and standards can be read either spectrophotometrically or fluorometrically. The MDA content in unknown samples is determined by comparison with the predetermined MDA standard curve.

Related Products

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
3. STA-816: OxiSelect™ N-epsilon-(Carboxymethyl) Lysine (CML) Competitive ELISA Kit
4. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
5. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit

Kit Components (shipped at room temperature)

1. MDA Standard (Part No. 233001): One 1 mL amber vial of 1.0 mM Malondialdehyde bis (dimethyl acetal)
2. Thiobarbituric Acid (TBA) (Part No. 233002): One 1 g bottle
3. SDS Lysis Solution (Part No. 233003): One 20 mL bottle
4. 2X TBA Acid Diluent (Part No. 233004): One 25 mL bottle
5. Sodium Hydroxide Solution (Part No. 233005): One 5 mL bottle
6. 100X BHT Solution (Part No. 233006): One 1 mL vial of 5% Butylated hydroxytoluene (BHT) in methanol

Materials Not Supplied

1. MDA samples: plasma, serum, urine, tissue or cell lysate
2. 1X PBS
3. n-Butanol
4. 96-well clear, flat-bottomed microplate for reading samples/standards
5. 96-well black fluorescence microplate for reading samples/standards

Storage

Store all components at 4ºC.

Preparation of Reagents

- 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 redissolve 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 (e.g.: 130 mg of TBA is enough to prepare 100 tests). Add 1X TBA Acid Diluent (see above) to the TBA and stir or mix vigorously until the powder has dissolved (e.g.: 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.

Preparation of Samples

Important Note: All samples should be assayed immediately upon collection or stored at -80°C for up to 1-2 months.

- Tissue: Because hemoglobin interferes with the assay, blood should be removed from tissue sample by perfusion with PBS containing heparin. Resuspend tissue at 50 to 100 mg/mL in PBS. To prevent further oxidation, add 100X BHT Solution to achieve a final concentration of 1X (for example, add 10 µL of 100X BHT to 1 mL of sample volume). Homogenize the tissue sample on ice, spin at 10,000 g for 5 min to collect the supernatant. The supernatant can be assayed directly for its TBARS level and results can be normalized based on its protein concentration.
- Plasma: To minimize the hemoglobin interference, prepare the plasma sample as soon as possible after blood being drawn. 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). Plasma samples can be assayed directly without further processing.
- Cells: Resuspend cells at 1-2 x 10^7 cells/mL in PBS. To prevent further oxidation, add 100X BHT Solution to achieve a final concentration of 1X (for example, add 10 µL of 100X BHT to 1 mL of cell suspension). Homogenize or sonicate the cells on ice. Use the whole homogenate in the assay.
- Urine: To remove insoluble particles, spin at 10,000 g for 5 min. The supernatant can be assayed directly.

Preparation of Standard Curve

Prepare a dilution series of MDA standards in the concentration range of 125 µM – 0 µM by diluting the MDA Standard in distilled or deionized water (Table 1). It is recommended that standards be performed in duplicate.

Standard Tubes	MDA Standard (µL)	Water (µL)	MDA Standard (µM)
1	125µL	875µL	125
2	250µL of Tube #1	250µL	62.5
3	250µL of Tube #2	250µL	31.25
4	250µL of Tube #3	250µL	15.63
5	250µL of Tube #4	250µL	7.81
6	250µL of Tube #5	250µL	3.91
7	250µL of Tube #6	250µL	1.95
8	250µL of Tube #7	250µL	0.98
9	0µL	250µL	0.0

Table 1. Preparation of MDA Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each MDA-containing sample and standard should be assayed in duplicate. High content MDA samples can be further diluted for analysis.
2. Add 100 µL of unknown samples or MDA standards to separate microcentrifuge tubes.

3. 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.
4. Add 250 µL of TBA Reagent to each sample and standard to be tested.
5. Close each tube and incubate at 95°C for 45-60 minutes.
6. Remove tubes and cool to room temperature in an ice bath for 5 minutes.
7. Centrifuge all sample tubes at 3000 rpm for 15 minutes. Remove the supernatant from samples for further analysis.
8. (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 (Step 7) to another tube, add 300 µL of n-Butanol. Vortex vigorously for 1-2 minutes and centrifuge for 5 minutes at 10,000 g.
   b. Transfer the butanol fraction for further measurement.
9. 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.

   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.

Example of Results

The following figures demonstrate typical MDA Quantitation results by TBARS Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

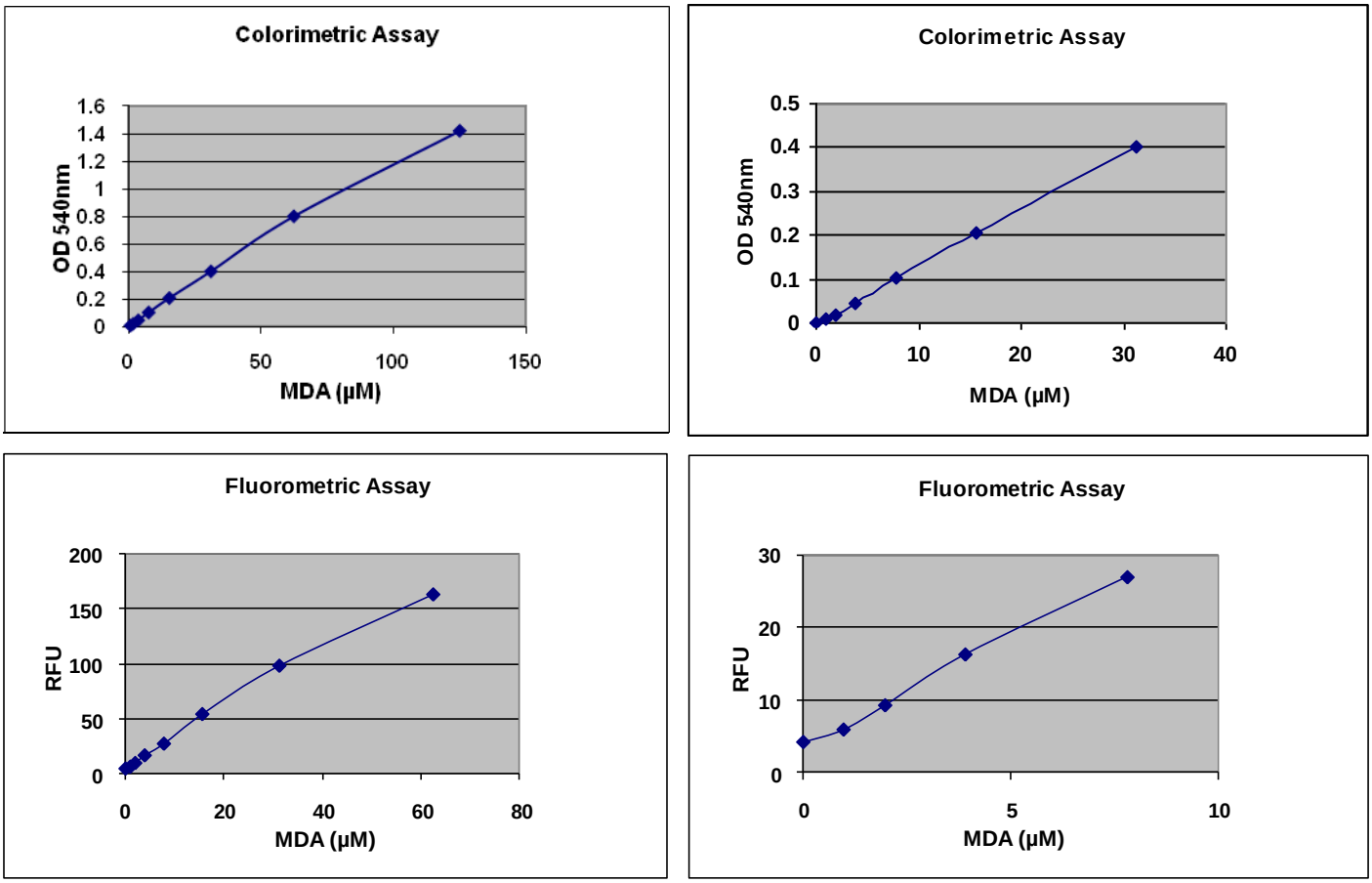

Figure 2. MDA Standard Curve. The MDA standard curve was created as described in the Assay Protocol. Top Panel: Colorimetric Detection; Bottom Panel: Fluorometric Detection.

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Recent Product Citations

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