Datasheet for ABIN2344986

OxiSelect™ TBARS Assay Kit (MDA Quantitation)

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Overview

Quantity:	200 tests
Reactivity:	Others
Application:	Immunoassay (IA), Quantification (Q)

Product Details

Purpose:	The Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit is a tool for the direct quantitative measurement of MDA in biological samples.
Brand:	OxiSelect™
Sample Type:	Serum, Plasma, Urine
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Characteristics:	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.
Components:	1. MDA Standard : One amber vial of 1.0 mM Malondialdehyde bis(dimethyl acetal) - 1.0 mL 2. Thiobarbituric Acid (TBA) : One bottle - 1.0 gram 3. SDS Lysis Solution : One bottle - 20 mL 4. 2X TBA Acid Diluent : One bottle - 25 mL 5. Sodium Hydroxide Solution : One bottle - 5 mL 6. 100X BHT Solution : One vial of 5% Butylated hydroxytoluene (BHT) in methanol - 1.0 mL
Material not included:	1. MDA samples: plasma, serum, urine, tissue or cell lysate

Product Details

2. Microcentrifuge and conical tubes
3. Heat block, incubator or water bath
4. n-Butanol
5. 96-well clear, flat-bottomed microplate for reading samples/standards
6. 96-well black fluorescence microplate for reading samples/standards 3
7. Spectrophotometric microplate reader capable of reading at 532nm
8. Fluorometric microplate reader capable of reading at 540nm excitation and 590nm emission

Target Details

Background:	Lipid peroxidation is a well-defined mechanism of cellular damage in animals and plants. 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. These aldehydic secondary products of lipid peroxidation are generally accepted markers of oxidative stress. 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. 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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Quick 30 minute assessment for oxidative stress • User friendly protocol uses smaller reaction volumes in a 96-well format

	• Does not require glass tubes or marbles
Assay Time:	0.5 h
Protocol:	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 2 be read either spectrophotometrically or fluorometrically. The MDA content in unknown samples is determined by comparison with the predetermined MDA standard curve.
Reagent Preparation:	• 1X TBA Acid Diluent: Dilute the 2X TBA Diluent 1:2 with 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 (eg: 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 (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. • 1X BHT Solution: Add antioxidant BHT at 1X final to each test sample to prevent further oxidation of lipid during sample processing and the TBA reaction.
Sample Preparation:	Important Note: All samples should be assayed immediately 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 containing 1X BHT. 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. Add 1X BHT to plasma sample to prevent further oxidation. Plasma sample can be assayed directly without further processing. • Cells: Resuspend cells at 1-2 x 107 cells/mL in PBS containing 1X BHT. 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. 4
Assay Procedure:	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.

Application Details

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 5 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.

Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Store all components at 4°C until their expiration dates.

Publications

Product cited in:

Yamamoto, Aizawa, Mieno, Karamatsu, Hirano, Furui, Miyashita, Yamazaki, Inakuma, Sato, Suganuma, Iwamoto: "The effects of tomato juice on male infertility." in: **Asia Pacific journal of clinical nutrition**, Vol. 26, Issue 1, pp. 65-71, (2017) (PubMed).

Chen, Yang, Hung, Ou, Pan, Cheng, Stern, Lo, Chiu et al.: "Impaired embryonic development in glucose-6-phosphate dehydrogenase-deficient Caenorhabditis elegans due to abnormal redox homeostasis induced activation of calcium-independent phospholipase and ..." in: **Cell death & disease**, Vol. 8, Issue 1, pp. e2545, (2017) (PubMed).

Fredriksson, Sellberg, Bowden, Engstrand, Berglund, Lilja: "Sutures impregnated with carbazate-activated polyvinyl alcohol reduce intraperitoneal adhesions." in: **Journal of pediatric surgery**, (2017) (PubMed).

Publications

Dowman, McDonald, Bozinovski, Vlahos, Gillies, Pouniotis, Hill, Goh, Holland: "Greater endurance capacity and improved dyspnoea with acute oxygen supplementation in idiopathic pulmonary fibrosis patients without resting hypoxaemia." in: **Respirology (Carlton, Vic.)**, (2017) (PubMed).

Wang, Li, Klaunig: "Investigation of the mechanism of triclosan induced mouse liver tumors." in: **Regulatory toxicology and pharmacology : RTP**, Vol. 86, pp. 137-147, (2017) (PubMed).

There are more publications referencing this product on: Product page

Images

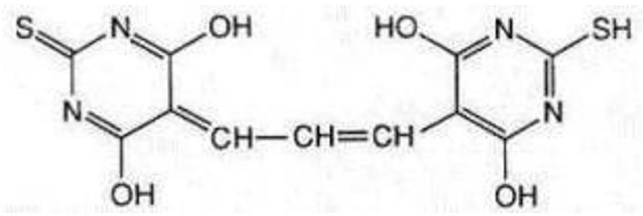
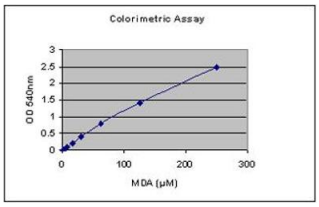
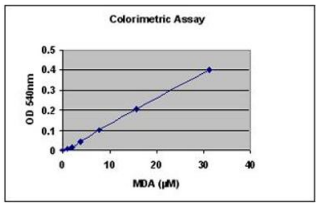


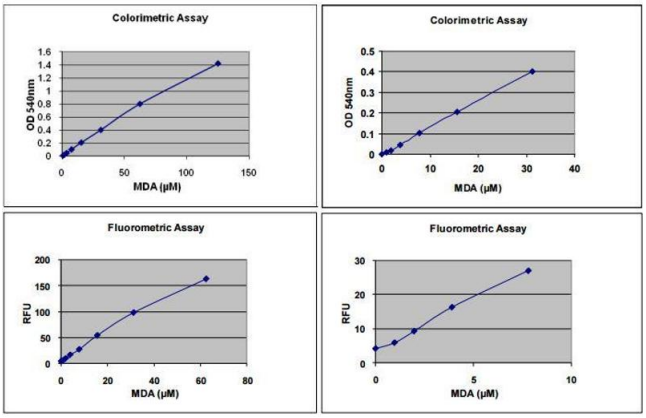
Image 1. MDA-TBA Adduct





Immunoassay

Image 2. OxiSelect™ TBARS Assay Kit Standard Curve with Colorimetric Detection. The MDA standard curve can also be generated using fluorometric detection.



Immunoassay

Image 3. MDA Standard Curve. The MDA standard curve was created as described in the Assay Protocol. Top Panel: Colorimetric Detection; Bottom Panel: Fluorometric Detection.