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.

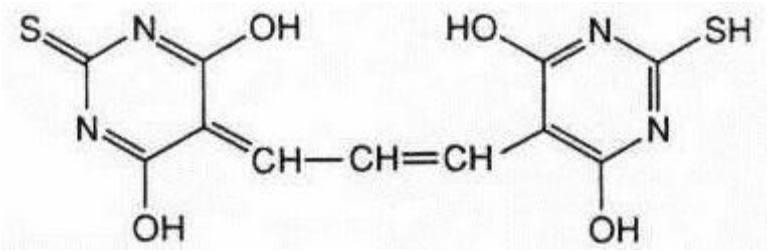

Figure 1. MDA-TBA Adduct

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

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.

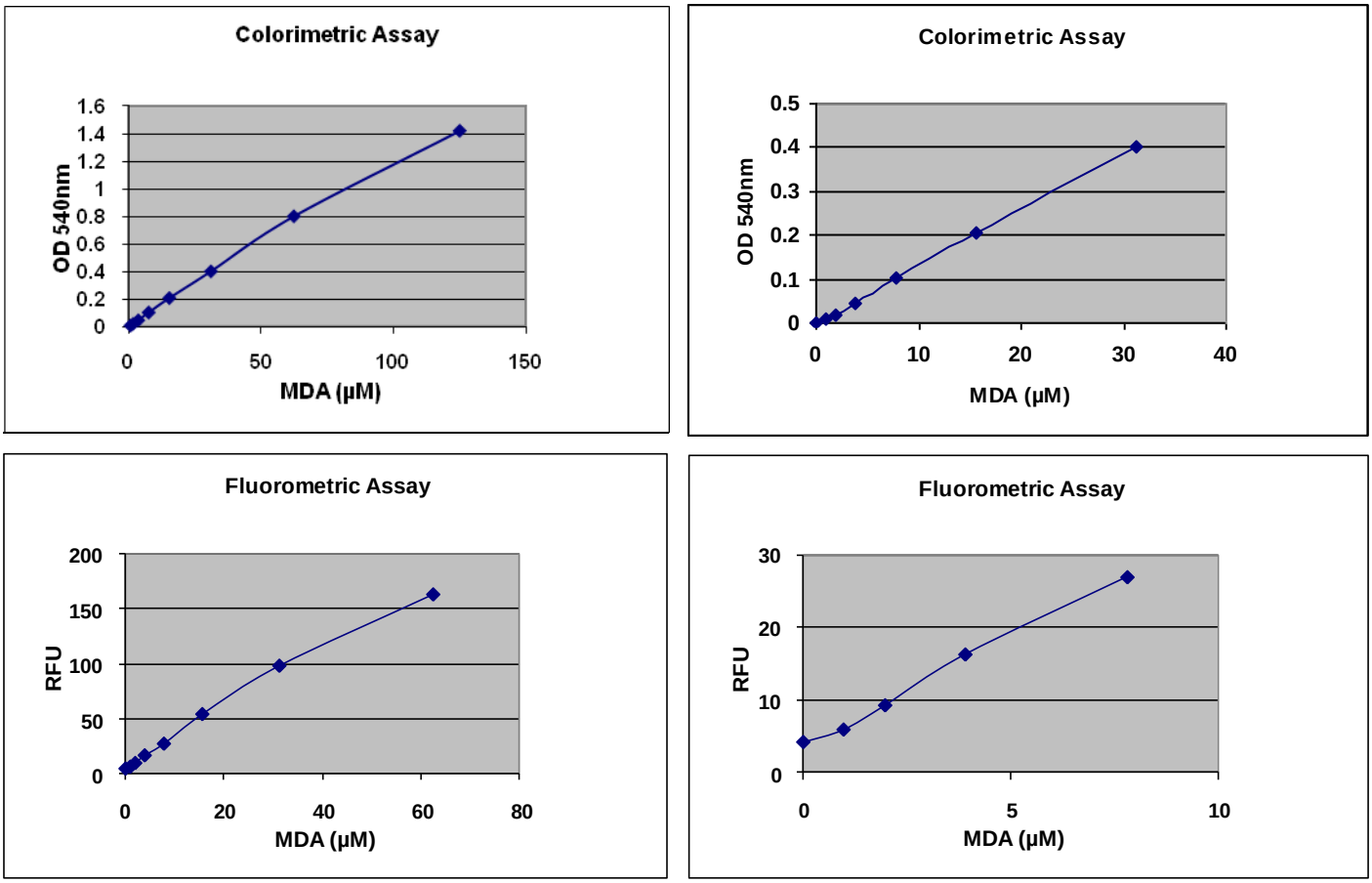

Figure 2. MDA Standard Curve. The MDA standard curve was created as described in the Assay Protocol. Top Panel: Colorimetric Detection; Bottom Panel: Fluorometric Detection.

References

1. Armstrong, D. and Browne, R. (1994). *Free Radicals in Diagnostic Medicine.* 366: 43-58.
2. Armstrong, D., et al. (1998). *Free Radicals and Antioxidant Protocols.* 108: 315-324.
3. Boyum, A. (1966). *J. of Clinical Investigation.* 21: Supplement 97.
4. Braun, D. and Fromherz, P. (1997). *Applied Physics A.*
5. Gidez, L., et al. (1982). *J. of Lipid Research.* 23: 1206-1223.
6. Lef'evre G., et al. (1998). *Annals de Biologie Clinique.* 56(3): 305-319.
7. Ohkawa, H., et al. (1979). *Anal. Biochem.* 95: 351-358.
8. Yagi, K. (1998). *Free Radicals and Antioxidant Protocols.* 108: 101-106.

Recent Product Citations

1. Nugroho, D. et al. (2023). Hepatoprotective effects of ethnic cabbage dishes: a comparison study on kimchi and pao cai. *J. Ethn. Food*. **10**(31). doi: 10.1186/s42779-023-00201-7.
2. Dewidar, B. et al. (2023). Alterations of hepatic energy metabolism in murine models of obesity, diabetes and fatty liver diseases. *EBioMedicine*. **94**:104714. doi: 10.1016/j.ebiom.2023.104714.
3. Deng, Z. et al. (2023). Efficacy of soy protein concentrate replacing animal protein supplements in mucosa-associated microbiota, intestinal health, and growth performance of nursery pigs. *Animal Nutrition*. doi: 10.1016/j.aninu.2023.06.007.

4. Deng, Z. et al. (2023). Comparative effects of soy protein concentrate, enzyme-treated soybean meal, and fermented soybean meal replacing animal protein supplements in feeds on growth performance and intestinal health of nursery pigs. *J Anim Sci Biotechnol*. **14**(1):89. doi: 10.1186/s40104-023-00888-3.
5. Abdallah, M. S. et al. (2023). Therapeutic Management, Clinicopathological, Molecular and Cost Studies on Sarcoptes scabiei Infestation in Rabbit. *J. Adv. Vet*. **13**(3):333-338.
6. Won, S.Y. et al. (2023). Effect of individual or combination of dietary betaine and glycine on productive performance, stress response, liver health, and intestinal barrier function in broiler chickens raised under heat stress conditions. *Poult Sci*. **102**(7):102771. doi: 10.1016/j.psj.2023.102771.
7. Hemraj, D. A. et al. (2023). Acidification and hypoxia drive physiological trade-offs in oysters and partial loss of nutrient cycling capacity in oyster holobiont. *Front Ecol Evol*. doi: 10.3389/fevo.2023.1083315.
8. Mayer, W. et al. (2023). Biomolecules of Fermented Tropical Fruits and Fermenting Microbes as Regulators of Human Hair Loss, Hair Quality, and Scalp Microbiota. *Biomolecules*. **13**(4):699. doi: 10.3390/biom13040699.
9. Leanza, G. et al. (2023). Oxidative Stress in Postmenopausal Women with or without Obesity. *Cells*. **12**(8):1137. doi: 10.3390/cells12081137.
10. Nikolic, A. et al. (2023). Chronic stress targets mitochondrial respiratory efficiency in the skeletal muscle of C57BL/6 mice. *Cell Mol Life Sci*. **80**(4):108. doi: 10.1007/s00018-023-04761-4.
11. Šķesters, A. et al. (2023). Selenium Status and Oxidative Stress in SARS-CoV-2 Patients. *Medicina (Kaunas)*. **59**(3):527. doi: 10.3390/medicina59030527.
12. Baik, K. Y. et al. (2023). Synergistic Effect of Hydrogen Peroxide and Cold Atmospheric Pressure Plasma-Jet for Microbial Disinfection. *Applied Sciences*. **13**(5):3324. doi: 10.3390/app13053324.
13. Peris-Martínez, C. et al. (2023). Antioxidant and Anti-Inflammatory Effects of Oral Supplementation with a Highly-Concentrated Docosahexaenoic Acid (DHA) Triglyceride in Patients with Keratoconus: A Randomized Controlled Preliminary Study. *Nutrients*. **15**(5):1300. doi: 10.3390/nu15051300.
14. Dos Anjos, C. et al. (2023). New Insights into the Bacterial Targets of Antimicrobial Blue Light. *Microbiol Spectr*. **11**(2): e0283322. doi: 10.1128/spectrum.02833-22.
15. Jack, B.U. et al. (2023). Cyclopia intermedia (Honeybush) Induces Uncoupling Protein 1 and Peroxisome Proliferator-Activated Receptor Alpha Expression in Obese Diabetic Female db/db Mice. *Int J Mol Sci*. **24**(4):3868. doi: 10.3390/ijms24043868.
16. Hashida, M. et al. (2023). α-Tocopherol Transfer Protein-Null Mice with Very Low α-Tocopherol Status Do Not Have an Enhanced Lipopolysaccharide-Induced Acute Inflammatory Response. *Curr Dev Nutr*. doi: 10.1016/j.cdnut.2022.100017.
17. Lee, J.I. et al. (2023). Transcriptomic and phenotypic changes of Cronobacter sakazakii ATCC 29544 grown under desiccation stress. *LWT*. doi: 10.1016/j.lwt.2022.114279.
18. Jeon, H.J. et al. (2023). Developmental toxicity of chlorpyrifos-methyl and its primary metabolite, 3,5,6-trichloro-2-pyridinol to early life stages of zebrafish (Danio rerio). *Ecotoxicol Environ Saf*. doi: 10.1016/j.ecoenv.2022.114352.
19. Xu, X. et al. (2022). Postbiotics effects of Lactobacillus fermentate on intestinal health, mucosa-associated microbiota, and growth efficiency of nursery pigs challenged with F18 + Escherichia coli. *J Anim Sci*. doi: 10.1093/jas/skac210.
20. Yun, Y.S. et al. (2022). Inactivation of Foodborne Pathogens on Inshell Walnuts by UV-C Radiation. *J Food Prot*. doi: 10.4315/JFP-21-442.

21. Kosutova, P. et al. (2022). Time-Dependent Oxidative Alterations in Plasma and Lung Tissue after Meconium Aspiration in a Rabbit Model. *Antioxidants (Basel)*. **12**(1):37. doi: 10.3390/antiox12010037.
22. Tanawattanasuntorn, T. et al. (2022). Trans-(±)-Kusunokinin Binding to AKR1B1 Inhibits Oxidative Stress and Proteins Involved in Migration in Aggressive Breast Cancer. *Antioxidants (Basel)*. **11**(12):2347. doi: 10.3390/antiox11122347.
23. Ryu, J.H. et al. (2022). Fermented and Aged Ginseng Sprouts (Panax ginseng) and Their Main Component, Compound K, Alleviate Asthma Parameters in a Mouse Model of Allergic Asthma through Suppression of Inflammation, Apoptosis, ER Stress, and Ferroptosis. *Antioxidants*. **11**(10):2052. doi: 10.3390/antiox11102052.
24. Lee, J.H. et al. (2022). Evaluation of tryptophan biomass as an alternative to conventional crystalline tryptophan in broiler diets. *J Appl Poult Res*. doi: 10.1016/j.japr.2022.100302.
25. Kim, Y.S. et al. (2022). Fermented Laminaria japonica improves working memory and antioxidant defense mechanism in healthy adults: a randomized, double-blind, and placebo-controlled clinical study. *Fish Aquat Sci*. **25**(8):450-461. doi: 10.47853/FAS. 2022.e41.
26. Rajab, B.S. et al. (2022). Antioxidative and Anti-Inflammatory Protective Effects of β-Caryophyllene against Amikacin-Induced Nephrotoxicity in Rat by Regulating the Nrf2/AMPK/AKT and NF-κB/TGF-β/KIM-1 Molecular Pathways. *Oxid Med Cell Longev*. doi: 10.1155/2022/4212331.
27. Kushwah, A.S. et al. (2022). Cardioprotective Activity of Cassia fistula L. Bark Extract in Isoproterenol-Induced Myocardial Infarction Rat Model. *Evid Based Complement Alternat Med*. doi: 10.1155/2022/6874281.
28. Deng, Z. et al. (2022). Soy protein concentrate replacing animal protein supplements and its impacts on intestinal immune status, intestinal oxidative stress status, nutrient digestibility, mucosa-associated microbiota, and growth performance of nursery pigs. *J Anim Sci*. doi: 10.1093/jas/skac255.
29. Vasavda, C. et al. (2022). Identification of the NRF2 transcriptional network as a therapeutic target for trigeminal neuropathic pain. *Sci Adv*. **8**(31): eabo5633. doi: 10.1126/sciadv. abo5633.
30. Navarro, J.A. et al. (2022). Endocrine and Metabolic Impact of Oral Ingestion of a Carob-Pod-Derived Natural-Syrup-Containing D-Pinitol: Potential Use as a Novel Sweetener in Diabetes. *Pharmaceutics*. **14**(8):1594. doi: 10.3390/pharmaceutics14081594.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com