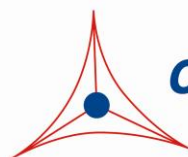

Product Manual

OxiSelect™ MDA Adduct Competitive ELISA Kit

Catalog Number

STA-832	96 assays
STA-832-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

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.

Both MDA and HNE have been shown to be capable of binding to proteins and forming stable adducts, also termed advanced lipid peroxidation end products. These modifications of proteins by MDA or HNE can cause both structural and functional changes of oxidized proteins.

The OxiSelect™ MDA Adduct Competitive ELISA Kit is an enzyme immunoassay developed for rapid detection and quantitation of MDA-protein adducts. The quantity of MDA adduct in protein samples is determined by comparing its absorbance with that of a known MDA-BSA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

Assay Principle

First, an MDA conjugate is coated on an ELISA plate. The unknown MDA protein samples or MDA - BSA standards are then added to the MDA conjugate preabsorbed ELISA plate. After a brief incubation, an anti-MDA polyclonal antibody is added, followed by an HRP conjugated secondary antibody. The content of MDA protein adducts in unknown samples is determined by comparison with a predetermined MDA-BSA standard curve.

Related Products

1. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
2. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
3. STA-811: OxiSelect™ Methylglyoxal (MG) Competitive ELISA Kit
4. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
5. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-MDA Antibody (1000X) (Part No. 283201): One 10 μ L vial of anti-MDA Antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231009): One 20 μ L vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. MDA-BSA Standard (Part No. 283202): One 50 μ L vial of 1 mg/mL MDA-BSA in PBS at 240 nmol MDA/mg proteins. The amount of MDA adduct is predetermined by a TBARS assay kit (Cat. # STA-330).
2. MDA Conjugate (Part No. 283203): One 20 μ L vial of 1.0 mg/mL MDA conjugate in PBS.
3. 100X Conjugate Diluent (Part No. 281603): One 300 μ L vial.

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
4. 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Anti-MDA Antibody, MDA-BSA Standard, MDA Conjugate and 100X Conjugate Diluent at -20°C to avoid multiple freeze/thaw cycles. Store all other kit components at 4°C.

Preparation of Reagents

- MDA Conjugate Coated Plate:

Note: The MDA Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 100 μ L to 9.9 mL of 1X PBS.

2. Immediately before use, prepare 500 ng/mL of MDA Conjugate by diluting the 1.0 mg/mL MDA Conjugate in 1X Conjugate Diluent. Example: Add 5 μ L of 1.0 mg/mL MDA Conjugate to 9.995 mL of 1X Conjugate Diluent and mix well.
 3. Add 100 μ L of the **500 ng/mL** MDA Conjugate to each well and incubate overnight at 4°C. Remove the MDA Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 μ L of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4°C and remove the Assay Diluent **immediately before use**.
- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
 - Anti-MDA Antibody and Secondary Antibody: Immediately before use, dilute the Anti-MDA antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of MDA-BSA standards in the concentration range of 0 to 6.25 μ g/mL by diluting the MDA-BSA Standard in Assay Diluent (Table 1).

Standard Tubes	1 mg/mL MDA-BSA Standard (μ L)	Assay Diluent (μ L)	MDA-BSA (μ g/mL)	MDA Adduct (pmol/mL)
1	5	795	6.25	1500
2	200 of Tube #1	200	3.13	750
3	200 of Tube #2	200	1.56	375
4	200 of Tube #3	200	0.78	188
5	200 of Tube #4	200	0.39	94
6	200 of Tube #5	200	0.20	47
7	200 of Tube #6	200	0.10	24
8	200 of Tube #7	200	0.05	12
9	200 of Tube #8	200	0.025	6
10	0	200	0	0

Table 1. Preparation of MDA-BSA Standards

Assay Protocol

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

1. Prepare and mix all reagents thoroughly before use. Each MDA sample including unknown and standard should be assayed in duplicate.

2. Add 50 μL of unknown sample or MDA-BSA standard to the wells of the MDA Conjugate coated plate. If needed, unknown samples may be diluted in 1X PBS containing 0.1% BSA before adding. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 μL of the diluted anti-MDA antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250 μL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 μL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 4 above.
6. Warm Substrate Solution to room temperature. Add 100 μL of Substrate Solution to each well. Incubate at room temperature for 2-30 minutes on an orbital shaker.
Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
7. Stop the enzyme reaction by adding 100 μL of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical MDA Adduct Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

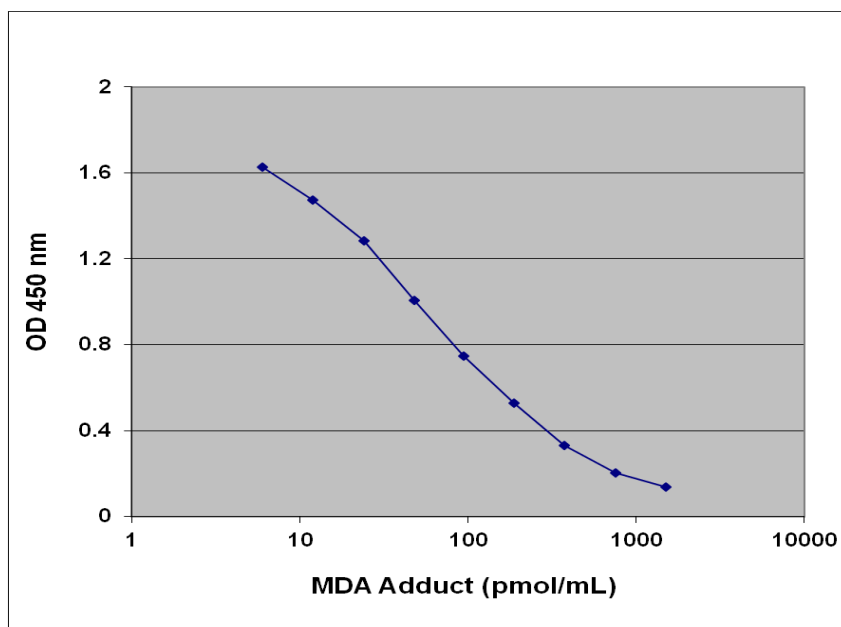


Figure 1: MDA-BSA Competitive ELISA Standard Curve

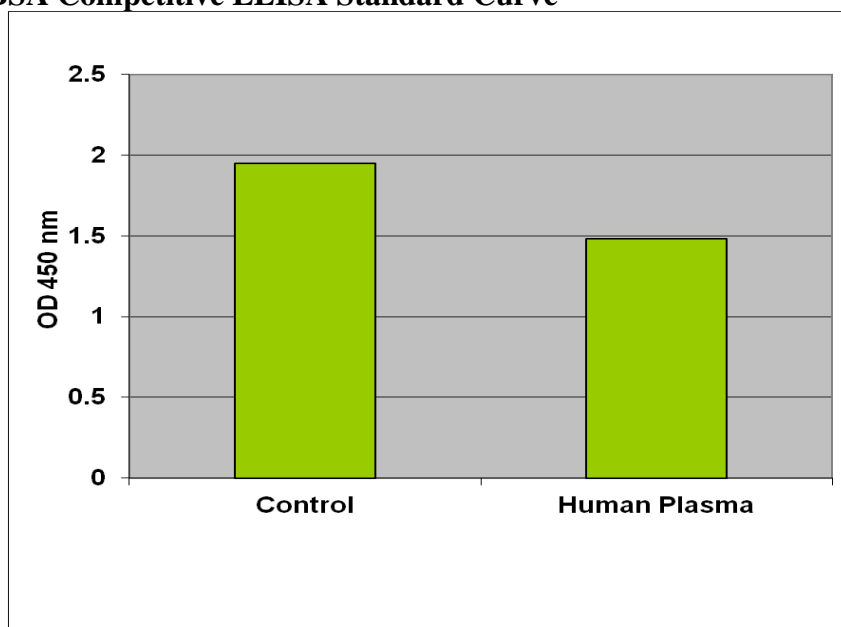


Figure 2: MDA Protein Adduct in Human Plasma. MDA levels were assayed in the blank (no MDA, left) and in undiluted human plasma (right).

References

1. Hoff HF, O'Neil J. (1993) *J Lipid Res.* 34: 1209-17.
2. Armstrong, D. and Browne, R. (1994). *Free Radicals in Diagnostic Medicine.* 366: 43-58.
3. Armstrong, D., et al. (1998). *Free Radicals and Antioxidant Protocols.* 108: 315-324.
4. Boyum, A. (1966). *J. of Clinical Investigation.* 21: Supplement 97.
5. Braun, D. and Fromherz, P. (1997). *Applied Physics A.*
6. Gidez, L., et al. (1982). *J. of Lipid Research.* 23: 1206-1223.
7. Lef'evre G., et al. (1998). *Annals de Biologie Clinique.* 56(3): 305-319.
8. Ohkawa, H., et al. (1979). *Anal. Biochem.* 95: 351-358.
9. Yagi, K. (1998). *Free Radicals and Antioxidant Protocols.* 108: 101-106.

Recent Product Citations

1. Jo, M.J. et al. (2023). Impaired NRF2 Inhibits Recovery from Ischemic Reperfusion Injury in the Aging Kidney. *Antioxidants (Basel).* **12**(7):1440. doi: 10.3390/antiox12071440.
2. Yamada, K. et al. (2023). Retinal ferroptosis as a critical mechanism for the induction of retinchoroiditis during ocular toxoplasmosis. *Redox Biol.* **67**:102890. doi: 10.1016/j.redox.2023.102890.
3. Elrayess, A.R. et al. (2023). Biochemical, Histological and Ultrastructural Studies on the Effect of Citric acid Supplementation on Aflatoxins-intoxicated Japanese Quail. *J. Adv. Vet.* **13**(6):958-964.
4. Jang, K.B. et al. (2023). Efficacy of zinc glycinate reducing zinc oxide on intestinal health and growth of nursery pigs challenged with F18+ Escherichia coli. *J Anim Sci.* **101**:skad035. doi: 10.1093/jas/skad035.
5. Hadžić, Z. et al. (2023). Oxidative Stress and C-Reactive Protein as Salivary Biomarkers in Smokers with Periodontitis Stage III and IV After Non-Surgical Periodontal Therapy (A Pilot Study). *Acta Med. Mediterr.* **39**:947-953. doi: 10.19193/0393-6384_2023_4_131.
6. Mashiko, S. et al. (2023). Broad responses to chemical adducts shape the natural antibody repertoire in early infancy. *Sci Adv.* **9**(19):eade8872. doi: 10.1126/sciadv.ade8872.
7. Pérez-Peiró, M. et al. (2023). Nitrosative and Oxidative Stress, Reduced Antioxidant Capacity, and Fiber Type Switch in Iron-Deficient COPD Patients: Analysis of Muscle and Systemic Compartments. *Nutrients.* **15**(6):1454. doi: 10.3390/nu15061454.
8. Zhou, Z. et al. (2023). Fe-Fe Double-Atom Catalysts for Murine Coronavirus Disinfection: Nonradical Activation of Peroxides and Mechanisms of Virus Inactivation. *Environ Sci Technol.* **57**(9):3804-3816. doi: 10.1021/acs.est.3c00163.
9. Somayajulu, M. et al. (2023). Airborne Exposure of the Cornea to PM10 Induces Oxidative Stress and Disrupts Nrf2 Mediated Anti-Oxidant Defenses. *Int J Mol Sci.* **24**(4):3911. doi: 10.3390/ijms24043911.
10. Yang, K.J. et al. (2023). Inhibition of Xanthine Oxidase Protects against Diabetic Kidney Disease through the Amelioration of Oxidative Stress via VEGF/VEGFR Axis and NOX-FoxO3a-eNOS Signaling Pathway. *Int J Mol Sci.* **24**(4):3807. doi: 10.3390/ijms24043807.
11. Baek, E.B. et al. (2022). Eriochloa villosa Alleviates Progression of Benign Prostatic Hyperplasia in vitro and in vivo. *Res Rep Urol.* **14**:313-326. doi: 10.2147/RRU.S381713.
12. Zhang, Q. et al. (2021). Micelle silymarin supplementation to sows' diet from day 109 of gestation to entire lactation period enhances reproductive performance, and affects serum hormones and metabolites. *J Anim Sci.* doi: 10.1093/jas/skab354.

13. Qin, L. et al. (2021). Systemic Profiles of microRNAs, Redox Balance, and Inflammation in Lung Cancer Patients: Influence of COPD. *Biomedicines*. **9**(10):1347. doi: 10.3390/biomedicines9101347.
14. Malkov, A. et al. (2021). A β initiates brain hypometabolism, network dysfunction and behavioral abnormalities via NOX2-induced oxidative stress in mice. *Commun Biol*. **4**(1):1054. doi: 10.1038/s42003-021-02551-x.
15. Zhang, Y. et al. (2021). Neuroprotective effect of the somatostatin receptor 5 agonist L-817,818 on retinal ganglion cells in experimental glaucoma. *Exp Eye Res*. **204**:108449. doi: 10.1016/j.exer.2021.108449.
16. Satta, H. et al. (2021) Amelioration of hemodialysis-induced oxidative stress and fatigue with a hemodialysis system employing electrolyzed water containing molecular hydrogen. *Ren Replace Ther*. doi: 10.1186/s41100-021-00353-9.
17. Li, Y. et al. (2021). Blue Light Induces Impaired Autophagy through Nucleotide-Binding Oligomerization Domain 2 Activation on the Mouse Ocular Surface. *Int. J. Mol. Sci*. **22**(4):2015. doi: 10.3390/ijms22042015.
18. Dong, S. et al. (2021). Leukemia inhibitory factor protects photoreceptor cone cells against oxidative damage through activating JAK/STAT3 signaling. *Ann Transl Med*. **9**(2):152. doi: 10.21037/atm-20-8040.
19. Clark, D. et al. (2021). A Randomized Double-Masked Phase 2a Trial to Evaluate Activity and Safety of Topical Ocular Reproxalap, a Novel RASP Inhibitor, in Dry Eye Disease. *J Ocul Pharmacol Ther*. doi: 10.1089/jop.2020.0087.
20. Alfarisi, H.A.H. et al. (2020). Hepatoprotective Effects of a Novel Trihoney against Nonalcoholic Fatty Liver Disease: A Comparative Study with Atorvastatin. *The Scientific World Journal*. doi: 10.1155/2020/4503253.
21. Pacifici, F. et al. (2020). Prdx6 Plays a Main Role in the Crosstalk Between Aging and Metabolic Sarcopenia. *Antioxidants (Basel)*. **9**(4). pii: E329. doi: 10.3390/antiox9040329.
22. Yang, J. et al. (2020). Sorting nexin 1 loss results in increased oxidative stress and hypertension. *FASEB J*. doi: 10.1096/fj.201902448R.
23. Zhu, H. et al. (2020). Effect of Certain Quinones on Adenosine Triphosphate Level in Human Bladder Cancer Cells. *Indian J Pharm Sci*. **2020**:82(1)spl issue2;1-6.
24. Shimizu, Y. et al. (2020). Role of DJ-1 in Modulating Glycative Stress in Heart Failure. *J Am Heart Assoc*. **9**(4). doi: 10.1161/jaha.119.014691.
25. El-Boshy, M. et al. (2020). Vitamin D3 and calcium cosupplementation alleviates cadmium hepatotoxicity in the rat: Enhanced antioxidative and anti-inflammatory actions by remodeling cellular calcium pathways. *J Biochem Mol Toxicol*. doi: 10.1002/jbt.22440.
26. Ognik, K. et al. (2019). The effect of a rat diet without added Cu on redox status in tissues and epigenetic changes in the brain. *Annals of Animal Science*. doi: 10.2478/aoas-2019-0075.
27. Katz, G.M. et al. (2019). Effects of genetic transfection on calcium cycling pathways mediated by double-stranded adeno-associated virus in post-infarction remodeling. *J Thorac Cardiovasc Surg*. doi: 10.1016/j.jtcvs.2019.08.089.
28. Chiang, S.S. et al. (2019). Role of Camellia brevistyla (Hayata) Coh. Stuart Seed Pomace Extract on Hypertension and Vascular Function in L-NAME-Treated Mice. *J Food Sci*. doi: 10.1111/1750-3841.14913.
29. Du, Y. et al. (2019). Chlorinated effluent organic matter causes higher toxicity than chlorinated natural organic matter by inducing more intracellular reactive oxygen species. *Sci Total Environ*. **701**:134881. doi: 10.1016/j.scitotenv.2019.134881.

30. Xu, A. et al. (2019). Protective effect of lycopene on testicular toxicity induced by Benzo[a]pyrene intake in rats. *Toxicology*. doi: 10.1016/j.tox.2019.152301.

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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