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-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
3. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
4. STA-811: OxiSelect™ Methylglyoxal (MG) Competitive ELISA Kit
5. STA-813: OxiSelect™ N^{ε}-(carboxyethyl) lysine (CEL) Competitive ELISA Kit
6. STA-816: OxiSelect™ N^{ε}-(carboxymethyl) lysine (CML) Competitive ELISA Kit
7. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
8. 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 µL vial of anti-MDA Antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231009): One 20 µL 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 µL 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 µL vial of 1.0 mg/mL MDA conjugate in PBS.
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL 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 -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

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 µL 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 µL of 1.0 mg/mL MDA Conjugate to 9.995 mL of 1X Conjugate Diluent and mix well.
3. Add 100 µL of the **500 ng/mL** MDA Conjugate to each well and incubate overnight at 4ºC. Remove the MDA Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4ºC 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 µg/mL by diluting the MDA-BSA Standard in Assay Diluent (Table 1).

Standard Tubes	1 mg/mL MDA-BSA Standard (µL)	Assay Diluent (µL)	MDA-BSA (µg/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 2-20 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.

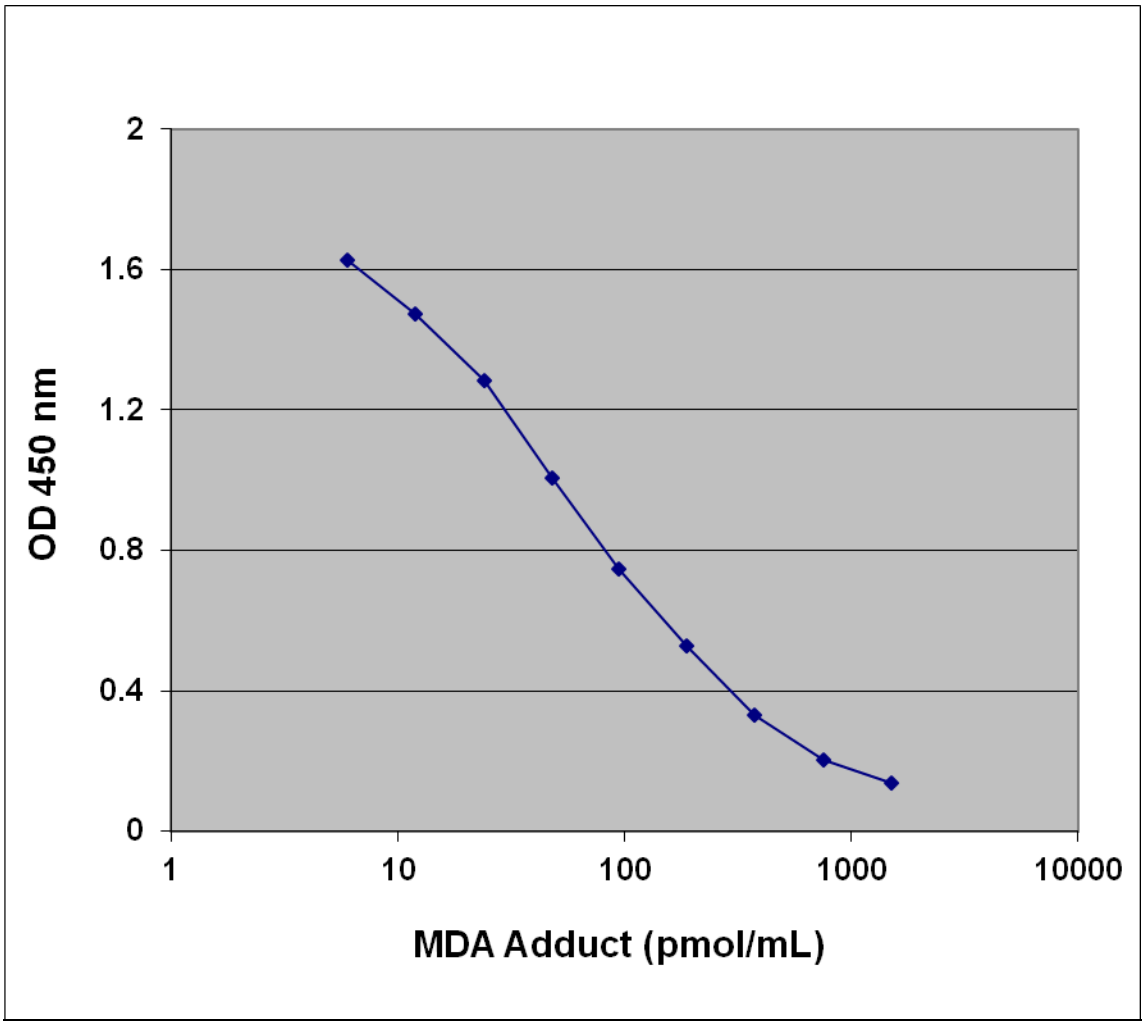

Figure 1: MDA-BSA Competitive ELISA Standard Curve

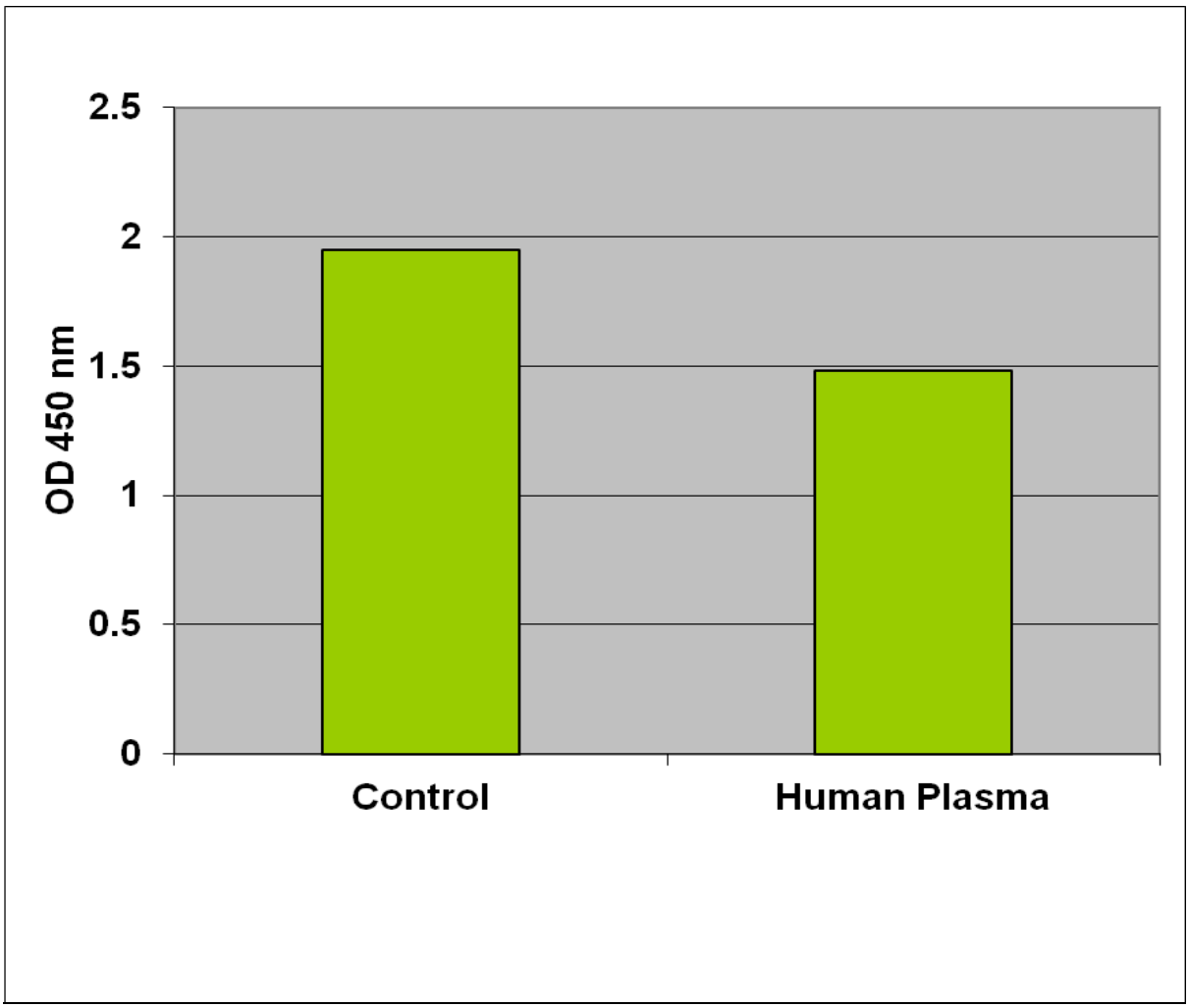

Figure 2: MDA Protein Adduct in Human Plasma. MDA levels were assayed in the blank (no MDA, left) and in undiluted human plasma (right).

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Recent Product Citations

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