Product Manual

OxiSelect™ Malondialdehyde (MDA) Immunoblot Kit

Catalog Number

STA-331 **10 blots**

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.

Cell Biolabs' MDA Immunoblot Kit is a simple and complete system for the detection of MDA-protein adducts. The kit includes antibodies for the detection of MDA in samples and an MDA-BSA Immunoblot Control for use as a positive control. Each kit provides sufficient quantities to perform at least 10 blots (7.5 cm x 8.5 cm).

Related Products

1. STA-303: OxiSelect™ Nitrotyrosine Immunoblot Kit
2. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
3. STA-308: OxiSelect™ Protein Carbonyl Immunoblot Kit
4. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
5. STA-315: OxiSelect™ Protein Carbonyl Spectrophotometric Assay
6. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
7. STA-332: OxiSelect™ MDA ELISA Kit
8. STA-334: OxiSelect™ HNE Adduct ELISA Kit
9. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG)
10. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG)

Kit Components

1. Rabbit Anti-MDA Antibody (Part No. 233101): One tube – 100 µL.
2. Goat Anti-Rabbit IgG, HRP-conjugate (Part No. 230802): One tube – 100 µL.
3. MDA-BSA Immunoblot Control (Part No. 233102): One tube – 100 µL of 1 µg/mL of MDA-BSA adduct (ready-to-use in 1X SDS-PAGE reducing sample buffer, pre-boiled).

Materials Not Supplied

1. Protein molecular weight standards
2. SDS-PAGE sample buffer
3. Polyacrylamide gels such as precast gels
4. Electrophoresis buffers
5. Electrophoresis and western blot transfer systems
6. Immunoblotting buffers such as TBST (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween-20)
7. PVDF or nitrocellulose membrane
8. Methanol
9. Non-fat dry milk
10. ECL reagents
11. Microcentrifuge tubes

Storage

Store all components at -20ºC. If the entire kit will not be used at once, aliquot kit components to avoid multiple freeze/thaw cycles.

Preparation of Reagents

- Rabbit Anti-MDA Antibody and Goat Anti-Rabbit IgG, HRP-conjugate: Immediately before use, dilute each Antibody 1:1000 with 5% non-fat dry milk/TBST. Do not store diluted solutions.

Assay Protocol

I. Electrophoresis and Transblotting

1. Prepare samples for electrophoresis with reducing SDS Sample Buffer.
2. Load 10 µL of MDA-BSA Immunoblot Control (provided pre boiled and ready-to-use) or prepared samples to wells of a polyacrylamide gel. It is recommended to include a pre-stained MW standard (as indicator of a successful transfer in step 3). Run the gel as per the manufacturer's instructions.
3. Transfer the gel proteins to a nitrocellulose or PVDF membrane as per the manufacturer's instructions.

II. Immunoblotting

1. After the transfer, remove the blot and wash once in TBST for 5 minutes.
2. Block the membrane with 5% non-fat dry milk in TBST for 1 hr at room temperature with constant agitation (blocking can also be performed overnight at 4°C).

3. Wash the blocked membrane three times with TBST, 5 minutes for each wash.
4. Incubate the membrane with the freshly diluted Rabbit Anti-MDA Antibody for 1-2 hr at room temperature with constant agitation (incubation can also be performed overnight at 4°C).
5. Wash the blotted membrane three times with TBST, 5 minutes for each wash.
6. Incubate the membrane with the freshly diluted Goat Anti-Rabbit IgG, HRP-conjugate for 1 hr at room temperature with constant agitation.
7. Wash the blotted membrane five times with TBST, 5 minutes for each wash.
8. Use the detection method of your choice. We recommend enhanced chemiluminescence reagents from Pierce.

Example of Results

The following figure demonstrates typical blot results for the MDA-BSA Immunoblot Control. One should use the data below for reference only. This data should not be used to interpret actual results.

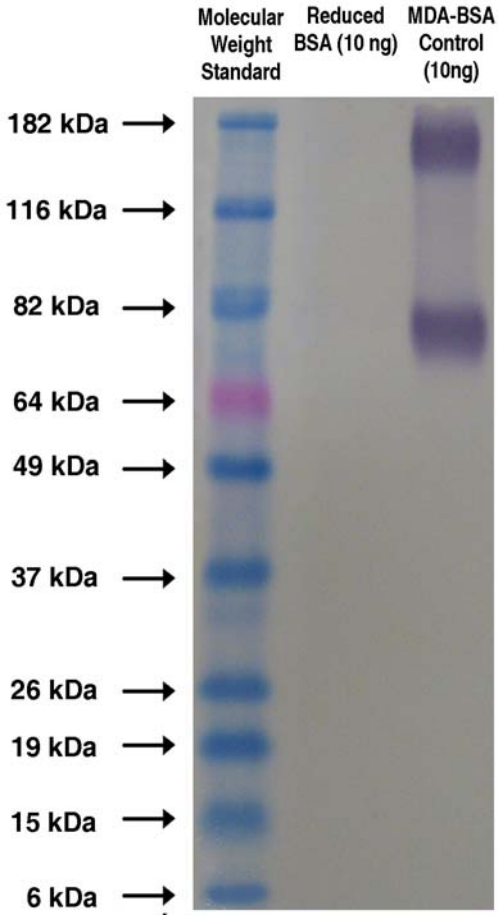

Figure 1: Immunoblotting of MDA-BSA Control. MDA-BSA Immunoblot Control, was first electroblotted onto nitrocellulose membrane. MDA was detected by immunoblotting with anti-MDA antibody as described in the Assay Protocol.

References

1. Bourdel-Marchasson, I. et al. 2001. *Age Aging* **30,** 235.
2. Kinalski, M., et al. 2000. *Acta Diabetol.* **37,** 179.

Recent Product Citations

1. Barabutis, N. et al. (2008). Antioxidant activity of growth hormone-releasing hormone antagonists in LNCaP human prostate cancer line. *PNAS* **105**:20470-20745.

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.

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