Product Manual

OxiSelect™ Malondialdehyde (MDA) Immunoblot Kit

Catalog Number

STA- 331 **10 blots**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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 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-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
3. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
4. STA-333: MDA-BSA Control
5. STA-337: OxiSelect™ 8-iso-Prostaglandin F2a Activity Assay Kit
6. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit
7. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
8. STA-816: OxiSelect™ N-epsilon-(Carboxymethyl) Lysine (CML) Competitive ELISA Kit
9. STA-817: OxiSelect™ Advanced Glycation End Products (AGE) Competitive ELISA Kit
10. STA-832: OxiSelect™ MDA Adduct Competitive ELISA Kit
11. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

1. Rabbit Anti-MDA Antibody (Part No. 233101): One tube – 100 µL.
2. Secondary Antibody, HRP-conjugate (Part No. 230805): 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 Secondary Antibody, HRP-conjugate: Immediately before use, dilute each Antibody 1:1000 with 5% non-fat dry milk/TBST. Do not store diluted solutions.

Assay Protocol

Important Note: MDA protein adducts are not stable long term. We recommend that all samples be tested fresh or after freezing at -80ºC for no more than one month.

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.

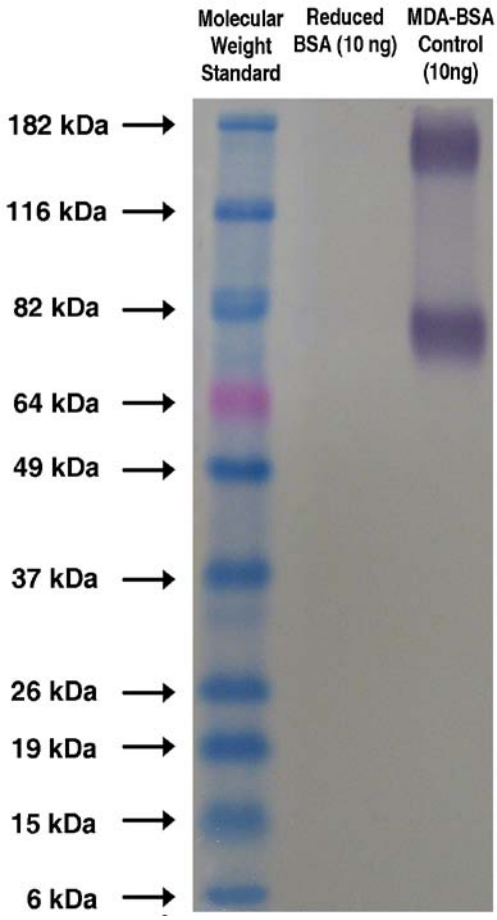

Figure 1: Immunoblotting of MDA-BSA Control. MDA-BSA Immunoblot Control, was first electroblotted onto nitrocellulose membrane. MDA was detected by immunoblotting with anti-MDA antibody as described in the Assay Protocol.

References

1. Bourdel-Marchasson, I. et al. 2001. *Age Aging* **30,** 235.
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Recent Product Citations

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6. Montez, P. et al. (2012). Angiotensin receptor blockade recovers hepatic UCP2 expression and aconitase and SDH activities and ameliorates hepatic oxidative damage in insulin resistant rats. *Endocrinology*. **153**:5845-5856.
7. Lazrak, A. et al. (2011). Regulation of alveolar epithelial Na+ channels by ERK1/2 in chlorine breathing mice. *Am. J. Respir. Cell Mol. Biol.* **46**:342-354.
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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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