Product Manual

OxiSelect™ HNE Adduct Competitive ELISA Kit

Catalog Number

STA-838	96 assays
STA-838-5	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. Specifically, 4-HNE can react with lysine, histidine or cysteine residues in protein to form adducts.

Cell Biolabs' OxiSelect™ HNE Adduct Competitive ELISA Kit is an enzyme immunoassay developed for rapid detection and quantitation of HNE protein adducts. The quantity of HNE adduct in protein samples is determined by comparing its absorbance with that of a known HNE-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 HNE conjugate is coated on an ELISA plate. The unknown HNE protein samples or HNE-BSA standards are then added to the HNE conjugate preabsorbed ELISA plate. After a brief incubation, an anti-HNE polyclonal antibody is added, followed by an HRP conjugated secondary antibody. The content of HNE protein adducts in unknown samples is determined by comparison with a predetermined HNE-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-832: OxiSelect™ MDA 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 (8 x 12).
2. Anti-HNE Antibody (1000X) (Part No. 283801): One 10 µL vial of anti-HNE antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231704): 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. HNE-BSA Standard (Part No. 283803): One 250 µL vial of 1 mg/mL HNE-BSA in PBS.
2. HNE Conjugate (Part No. 283802): One 50 µL vial of HNE conjugate at 1.0 mg/mL 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-HNE Antibody, HNE-BSA Standard, HNE Conjugate and 100X Conjugate Diluent at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Preparation of Reagents

- HNE Conjugate Coated Plate:

Note: The HNE 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 50 µL to 4.95 mL of 1X PBS.
2. Immediately before use, prepare 10 µg/mL of HNE Conjugate by diluting the 1.0 mg/mL HNE Conjugate in 1X PBS. Example: Add 25 µL to 2.475 mL of 1X PBS.

3. Mix the 10 µg/mL of HNE Conjugate and 1X Conjugate Diluent at 1:1 ratio and add 100 µL of the mixture to each well and incubate overnight at 4ºC. Remove the HNE 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 to 1X with deionized water. Stir to homogeneity.
- Anti-HNE Antibody and Secondary Antibody: Immediately before use, dilute the Anti-HNE 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 HNE-BSA standards in the concentration range of 0 to 200 µg/mL by diluting the HNE-BSA Standard in Assay Diluent (Table 1).

Standard Tubes	1 mg/mL HNE-BSA Standard (µL)	Assay Diluent (µL)	HNE-BSA (µg/mL)
1	80	320	200
2	200 of Tube #1	200	100
3	200 of Tube #2	200	50
4	200 of Tube #3	200	25
5	200 of Tube #4	200	12.5
6	200 of Tube #5	200	6.25
7	200 of Tube #6	200	3.13
8	200 of Tube #7	200	1.56
9	0	200	0

Table 1. Preparation of HNE-BSA Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each HNE sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or HNE-BSA standard to the wells of the HNE 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-HNE 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 HNE Adduct Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

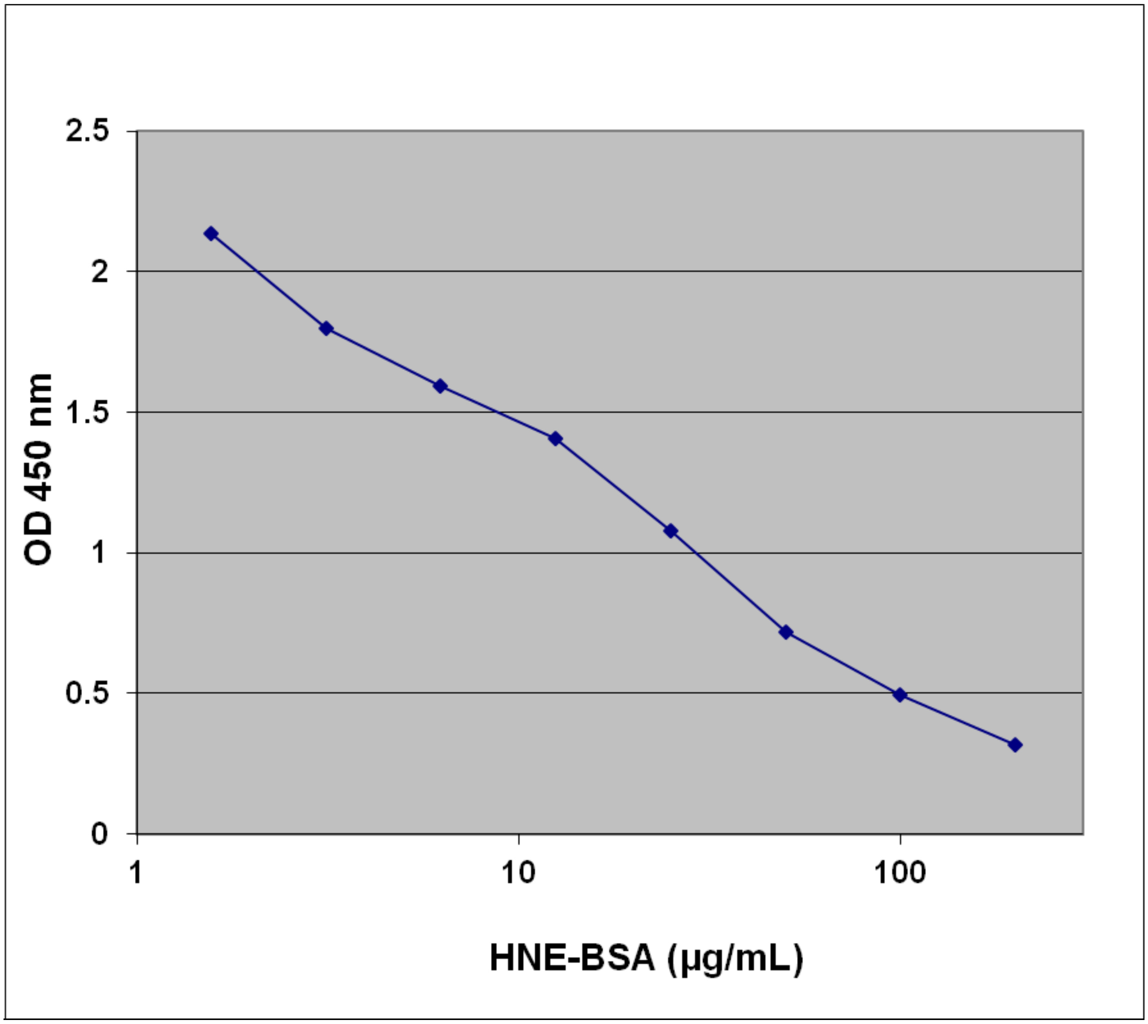

Figure 1: HNE-BSA Competitive ELISA Standard Curve.

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Recent Product Citations

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