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Product Manual

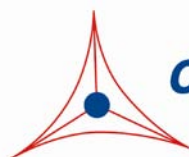
# OxiSelect™ HNE-His Adduct ELISA Kit

## Catalog Number

STA-334	96 assays
STA-334-5	5 x 96 assays

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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**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' OxiSelect™ HNE-His Adduct ELISA Kit is an enzyme immunoassay developed for rapid detection and quantitation of HNE-His protein adducts. The quantity of HNE-His 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**

BSA standards or protein samples (10 µg/mL) are adsorbed onto a 96-well plate for 2 hrs at 37°C. The HNE-protein adducts present in the sample or standard are probed with an anti-HNE-His antibody, followed by an HRP conjugated secondary antibody. The HNE-protein adducts content in an unknown sample is determined by comparing with a standard curve that is prepared from predetermined HNE-BSA standards.

## **Related Products**

1. STA-316: OxiSelect™ N-epsilon-(Carboxymethyl) Lysine (CML) ELISA Kit
2. STA-317: OxiSelect™ Advanced Glycation End Products (AGE) ELISA
3. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
4. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
5. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
6. STA-331: OxiSelect™ MDA Immunoblot Kit
7. STA-332: OxiSelect™ MDA Adduct ELISA Kit
8. STA-333: MDA-BSA Control
9. STA-335: HNE-BSA Control
10. STA-337: OxiSelect™ 8-iso-Prostaglandin F2a Activity Assay Kit
11. STA-344: OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit
12. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)

## **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-HNE-His Antibody (1000X) (Part No. 233401): One 20  $\mu$ L vial of anti-HNE-His Mouse IgG.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20  $\mu$ 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.
8. Reduced BSA Standard (Part No. 233202): One 500  $\mu$ L vial of 1 mg/mL reduced BSA in PBS.

### **Box 2 (shipped on blue ice packs)**

1. HNE-BSA Standard (Part No. 233402): One 150  $\mu$ L vial of 1 mg/mL HNE-BSA in PBS.

## **Materials Not Supplied**

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. 1X PBS
3. 10  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
4. 50  $\mu$ L to 300  $\mu$ 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 Reduced BSA and HNE-BSA Standards at -20°C to avoid multiple freeze/thaw cycles. Store all other kit components at 4°C.

## **Preparation of Reagents**

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate 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**

1. Freshly prepare 10 µg/mL of Reduced BSA and HNE-BSA Standards by diluting the 1 mg/mL BSA standards in 1X PBS. Example: Add 20 µL to 1.980 mL of 1X PBS.
2. Prepare a series of HNE-BSA standards according to Table 1.

Standard Tubes	10 µg/mL HNE-BSA (µL)	10 µg/mL Reduced BSA (µL)	HNE-BSA (µg/mL)
1	400	0	10
2	320	80	8.0
3	240	160	6.0
4	160	240	4.0
5	80	320	2.0
6	40	360	1.0
7	20	380	0.5
8	0	400	0

**Table 1. Preparation of HNE-BSA Standard Curve**

### **Assay Protocol**

1. Dilute unknown protein sample to 10 µg/mL in 1X PBS. Each protein sample and HNE-BSA Standard should be assayed in duplicate or triplicate.
2. Add 100 µL of the 10 µg/mL protein samples or Reduced/HNE-BSA standards to the 96-well Protein Binding Plate. Incubate at 37°C for at least 2 hours or 4°C overnight.

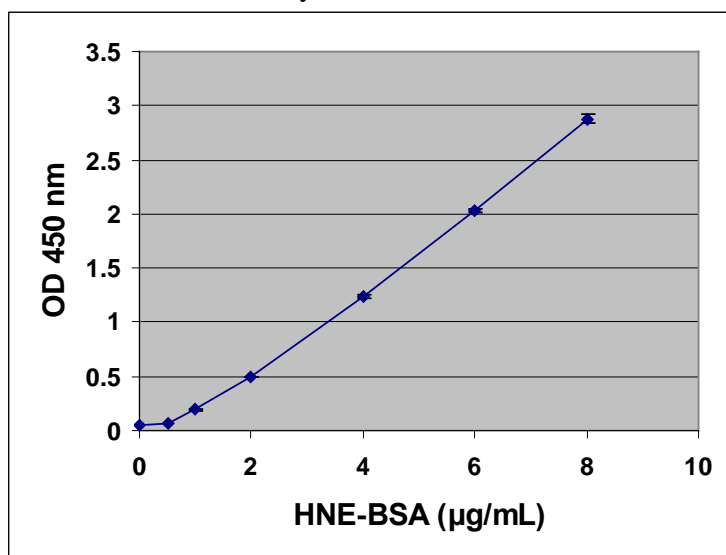
*Note: Lysate sample should not be prepared in lysis buffer containing Triton X-100, NP-40, or Igepal CA-630 because these detergents interfere with protein coating of the plate unless the detergent concentration in the 10 µg/mL protein samples is no more than 0.001%. We recommend lysis by homogenization or sonication.*

3. Wash wells 2 times with 250 µL 1X PBS per well. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash solution.
4. Add 200 µL of Assay Diluent per well and incubate for 1-2 hours at room temperature on an orbital shaker.

5. Wash 3 times with 250  $\mu$ L 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.
6. Add 100  $\mu$ L of the diluted Anti-HNE-His Antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 5 above.
7. Add 100  $\mu$ L 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 5 times according to step 5 above.
8. Warm Substrate Solution to room temperature. Add 100  $\mu$ L of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.  
*Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.*
9. Stop the enzyme reaction by adding 100  $\mu$ L of Stop Solution to each well. Results should be read immediately (color will fade over time).
10. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length. Use the Reduced BSA standard as absorbance blank.

### **Example of Results**

The following figures demonstrate typical HNE-His Adduct 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 ELISA Standard Curve.**

## **References**

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## **Recent Product Citations**

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## **Warranty**

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