Product Manual

OxiSelect™ HNE-His Adduct ELISA Kit

Catalog Number

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Number of Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>STA-334</td>
<td>96 assays</td>
</tr>
<tr>
<td>STA-334-5</td>
<td>5 x 96 assays</td>
</tr>
</tbody>
</table>

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.

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 µL vial of anti-HNE-His Mouse IgG.
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 µL 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 µL 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 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 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.

**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.

<table>
<thead>
<tr>
<th>Standard Tubes</th>
<th>10 μg/mL HNE-BSA (μL)</th>
<th>10 μg/mL Reduced BSA (μL)</th>
<th>HNE-BSA (μg/mL)</th>
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<tr>
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<td>0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
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<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>400</td>
<td>0</td>
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</table>

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 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.

6. Add 100 µL 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 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 5 times according to step 5 above.

8. Warm Substrate Solution to room temperature. Add 100 µL 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 µL 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.](image)
References


Recent Product Citations


**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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