Signosis, Inc.
Innovative Plate Assay Solutions

4-HNE Lipid Peroxidation ELISA Kit

Catalog # EA-7086 **(For Research Use Only)**

Introduction

4-Hydroxynonenal (4-HNE) is a major biomarker and bioactive byproduct of lipid peroxidation and oxidative stress. It is generated when reactive oxygen species attack polyunsaturated fatty acids within cell membranes, leading to the breakdown of lipid hydroperoxides and formation of reactive aldehydes such as 4-HNE. Elevated 4-HNE levels reflect oxidative damage to cellular lipids and indicate increased oxidative burden at both the cellular and systemic level. Because 4-HNE can form adducts with proteins, DNA, and mitochondria, it contributes to inflammation, impaired cellular signaling, and tissue dysfunction. Accumulation of 4-HNE has been associated with cardiovascular disease, neurodegeneration, metabolic disorders, and aging, making it a valuable marker for assessing oxidative injury and the balance of antioxidant defense systems.

Signosis' 4-HNE Lipid Peroxidation ELISA Kit can be used to analyze 4-HNE levels in serum, plasma, cell and tissue lysates, and other biological fluid samples.

Principle

Competitive ELISA

The 4-HNE assay uses the competitive ELISA format, which is ideal for small-molecule biomarkers lacking multiple epitopes for sandwich detection. Plates are pre-coated with 4-HNE-protein conjugate that competes with free 4-HNE in samples for binding to a specific anti-4-HNE antibody. After washing away unbound material, HRP reagent binds to the antibodies bound to the plate. TMB substrate generates a blue color proportional to bound antibody, turning yellow upon acidification. Absorbance at 450 nm is inversely proportional to 4-HNE concentration in the sample—higher free 4-HNE levels block the antibody from binding to the plate.

Materials Required but Not Provided

- Microplate reader capable of measuring absorbance at 450nm.
- 10N NaOH

Materials Provided

- 4-HNE ELISA Plate (4°C)
- Dilution Buffer (4°C)
- 5x Assay Wash Buffer (4°C)
- 4-HNE Standard (-20°C)
- Detection Antibody (-20°C)
- HRP Reagent (4°C)
- Substrate (4°C)
- Stop Solution (4°C)

****Spin down small tubes before starting experiment. ****

Urine Sample Preparation

1. Urine samples can be assayed directly or stored away at -80°C.

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Culture Medium Preparation

1. Cell culture medium can be assayed directly or stored away at -80°C.

Cell Lysate Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

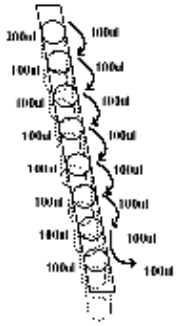
Assay Procedure

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O.
- Dilute Detection antibody 50 times with 1x Diluent buffer.
- Dilute HRP reagent 200 times with 1x Diluent buffer.

1. Standard curve preparation: Add 200 µL of 1x Diluent buffer to the first well of a strip and add 100 µL of 1x Diluent buffer to wells 2–8. Prepare a 100 ng/mL 4-HNE standard in the first well by mixing 2 µL of the provided 10 mg/mL 4-HNE standard with the 200 µL 1x Diluent buffer in the first well. Then, serially dilute 100 µL across wells 2–7 as shown in the diagram below. Make sure to discard 100 µL from the seventh, final well of the serial dilution. Leave the eight well untouched as a blank control.

Standard#	4-HNE Concentration (ng/mL)
1	100
2	50
3	25
4	12.5
5	6.25
6	3.125
7	1.5625
8	0



2. Add 100 µL of sample to each well of the ELISA plate and incubate for 1 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200 µL of 1x Assay wash buffer. Repeat the process three times for a total of three washes. Make sure all liquid is removed after each wash.
4. Add 100 µL of diluted Detection antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash step in step 3.
6. Add 100 µL of diluted HRP reagent to each well and incubate for 45 minutes at room temperature with gentle shaking.
7. Repeat the aspiration/wash step in step 3.
8. Add 100 µL of Substrate to each well and incubate for 10-30 minutes. **Substrate incubation time may vary due to differences in antibody reactivity. Stronger blue signals can be stopped earlier. Weaker signals can be incubated for a longer time up to 1 hour.**
9. Add 50 µL of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Immediately measure the absorbance of the plate at 450 nm using a plate reader.

4-HNE Standard Curve

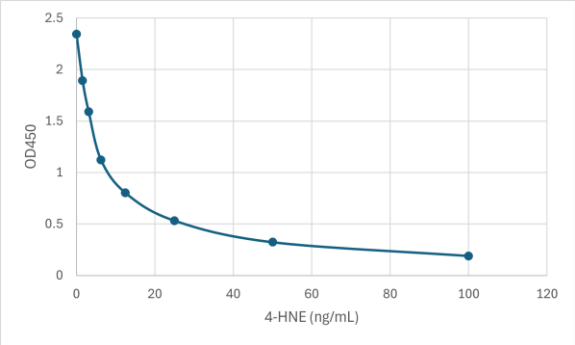


Figure 1. A 100 ng/mL 4-HNE standard was serially diluted two-fold on an 4-HNE–coated ELISA plate and quantified using a competitive ELISA with an anti–4-HNE antibody.