Signosis, Inc.
Innovative Plate Assay Solutions

Oxidative Damage ELISA Combo Kit for Fluids

Catalog # EA-7088 **(For Research Use Only)**

Introduction

Signosis' Oxidative Damage ELISA Combo Kit enables simultaneous measurement of three key oxidative stress biomarkers – 8-OHdG, 4-HNE, and oxLDL – in a single 96-well plate with 32 tests for each marker. 8-OHdG indicates oxidative DNA damage, 8-iso-PGF2α reflects lipid peroxidation, and OxLDL represents oxidized lipoproteins associated with inflammation and cardiovascular disease. This comprehensive multi-analyte panel reveals disease-specific oxidative damage patterns across DNA, lipids, and lipoproteins, providing a plate-based solution for assessing systemic oxidative injury in models of cardiovascular disease, metabolic disorders, neurodegeneration, aging, and therapeutic intervention studies.

This kit can be used to screen biological fluid samples including urine, plasma/serum, and cell media. For analyzing cell and tissue lysates, additional procedures are necessary for preparing the sample and we recommend using the single marker kits (EA-7085, EA-7086) for this purpose.

Principle

Competitive ELISA

The 8-OHdG and 4-HNE assays use the competitive ELISA format, which is ideal for small-molecule biomarkers lacking multiple epitopes for sandwich detection. Plates are pre-coated with marker-protein conjugates that compete with free markers in samples for binding to specific anti-marker antibodies. 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 marker concentration in the sample—higher free marker levels block the antibodies from binding to the plate.

Sandwich ELISA

OxLDL is measured using the sandwich ELISA format. OxLDL in the sample binds to capture antibodies pre-coated on the plate. After washing, biotinylated detection antibody binds to the captured OxLDL on the plate. HRP reagent and TMB substrate produce a blue color proportional to OxLDL concentration in the sample. Acidic stop solution turns the solution color yellow, and absorbance at 450 nm directly correlates with OxLDL levels in the sample.

Materials Required but Not Provided

- Microplate reader capable of measuring absorbance at 450nm.
- DNA Damage Extraction Kit (EA-7089)

Materials Provided

- Oxidative Damage ELISA Plate (4°C)
- Dilution Buffer (4°C)
- 5x Assay Wash Buffer (4°C)
- 8-OHdG Standard (-20°C)
- 4-HNE Standard (-20°C)
- OxLDL Standard (-20°C)
- Detection Antibody Mix (-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.

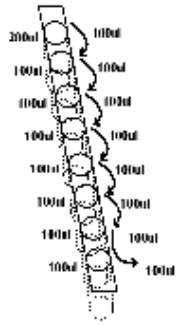
Standard Curve Preparation

If quantitative data is desired, a standard curve can be prepared for each marker (8-OHdG, 8-iso-PGF2α, OxLDL). Each curve will require an 8-well strip. If you only want to use the plate for profiling samples, you can skip the preparation of the standard curve and proceed with the measurement of your samples.

8-OHdG Standard Curve:

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 8-OHdG standard in the first well by mixing 2 µL of the provided 10 mg/mL 8-OHdG 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#	8-OHdG Concentration (ng/mL)
1	100
2	50
3	25
4	12.5
5	6.25
6	3.125
7	1.5625
8	0



8-OHdG Standard Curve

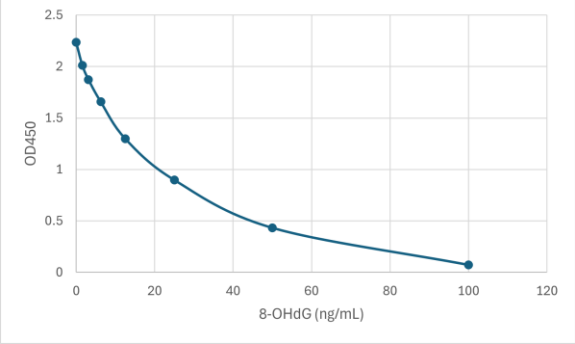
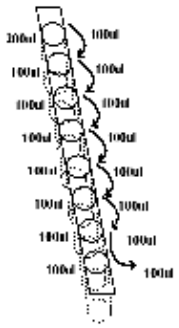


Figure 1. A 100 ng/mL 8-OHdG standard was serially diluted two-fold on an 8-OHdG–coated ELISA plate and quantified using a competitive ELISA with an anti–8-OHdG antibody.

4-HNE Standard Curve:
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



4-HNE Standard Curve

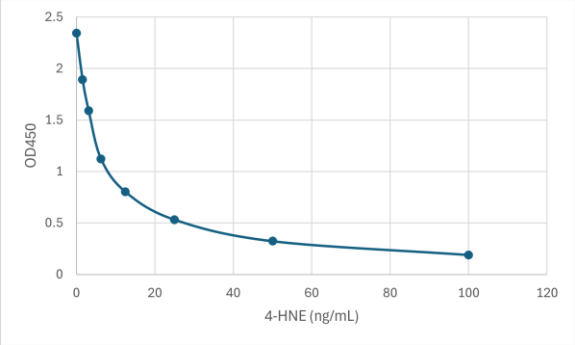
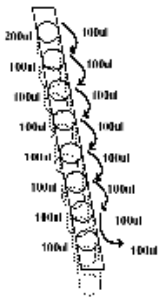


Figure 2. A 100 ng/mL 4-HNE standard was serially diluted two-fold on an 4-HNE–coated ELISA plate and quantified using a competitive ELISA with an anti–4-HNE antibody.

OxLDL Standard Curve:
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 50 ng/mL OxLDL standard in the first well by mixing 2 µL of the provided 5 mg/mL OxLDL 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#	OxLDL Concentration (ng/mL)
1	50
2	25
3	12.5
4	6.25
5	3.125
6	1.5625
7	0.78125
8	0



OxLDL Standard Curve

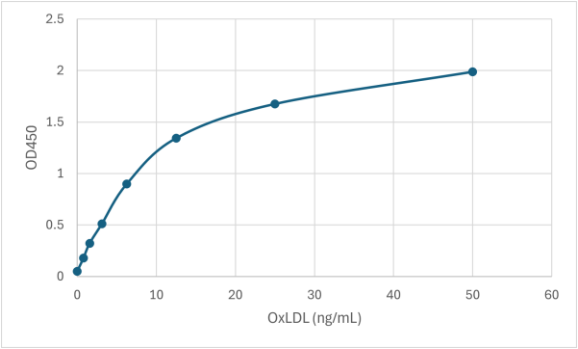


Figure 3. A 50 ng/mL OxLDL standard was serially diluted two-fold on an ELISA plate coated with anti-OxLDL capture antibody and quantified using a sandwich ELISA with an anti–OxLDL detection antibody.

Assay Procedure

Reagent preparation for experiment

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

1. Add 100 µL of sample to each well of the ELISA plate and incubate for 1 hour at room temperature with gentle shaking.
2. 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.
3. Add 100 µL of diluted Detection antibody mix to each well and incubate for 1 hour at room temperature with gentle shaking.
4. Repeat the aspiration/wash step in step 2.
5. Add 100 µL of diluted HRP reagent to each well and incubate for 45 minutes at room temperature with gentle shaking.
6. Repeat the aspiration/wash step in step 2.
7. 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.**
8. Add 50 µL of Stop solution to each well. The color in the wells should change from blue to yellow.
9. Immediately measure the absorbance of the plate at 450 nm using a plate reader.

Oxidative Damage ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	8-OHdG	8-OHdG	8-OHdG	8-OHdG	4-HNE	4-HNE	4-HNE	4-HNE	OxLDL	OxLDL	OxLDL	OxLDL
B	8-OHdG	8-OHdG	8-OHdG	8-OHdG	4-HNE	4-HNE	4-HNE	4-HNE	OxLDL	OxLDL	OxLDL	OxLDL
C	8-OHdG	8-OHdG	8-OHdG	8-OHdG	4-HNE	4-HNE	4-HNE	4-HNE	OxLDL	OxLDL	OxLDL	OxLDL
D	8-OHdG	8-OHdG	8-OHdG	8-OHdG	4-HNE	4-HNE	4-HNE	4-HNE	OxLDL	OxLDL	OxLDL	OxLDL
E	8-OHdG	8-OHdG	8-OHdG	8-OHdG	4-HNE	4-HNE	4-HNE	4-HNE	OxLDL	OxLDL	OxLDL	OxLDL
F	8-OHdG	8-OHdG	8-OHdG	8-OHdG	4-HNE	4-HNE	4-HNE	4-HNE	OxLDL	OxLDL	OxLDL	OxLDL
G	8-OHdG	8-OHdG	8-OHdG	8-OHdG	4-HNE	4-HNE	4-HNE	4-HNE	OxLDL	OxLDL	OxLDL	OxLDL
H	8-OHdG	8-OHdG	8-OHdG	8-OHdG	4-HNE	4-HNE	4-HNE	4-HNE	OxLDL	OxLDL	OxLDL	OxLDL