Signosis, Inc.
Innovative Plate Assay Solutions

OxLDL Oxidized Lipoprotein ELISA Kit

Catalog # EA-7087 **(For Research Use Only)**

Introduction

Oxidized low-density lipoprotein (OxLDL) is a major biomarker and mediator of oxidative stress–related vascular damage. It forms when reactive oxygen species modify native LDL particles, altering their structure and promoting inflammatory and atherogenic processes. Elevated OxLDL levels are closely linked to endothelial dysfunction, plaque formation, and cardiovascular disease progression. Monitoring OxLDL provides valuable insight into oxidative lipid damage, cardiovascular risk, and the effectiveness of antioxidant or lipid-lowering therapies.

Signosis' OxLDL Oxidized Lipoprotein ELISA Kit can be used to analyze OxLDL levels in serum, plasma, cell and tissue lysates, and other biological fluid samples.

Principle

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.

Materials Provided

- OxLDL ELISA Plate (4°C)
- Dilution Buffer (4°C)
- 5x Assay Wash Buffer (4°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. ****

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.

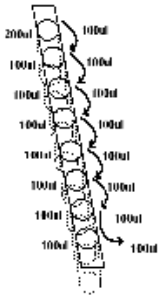
Assay Procedure

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 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#	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



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.

OxLDL Standard Curve

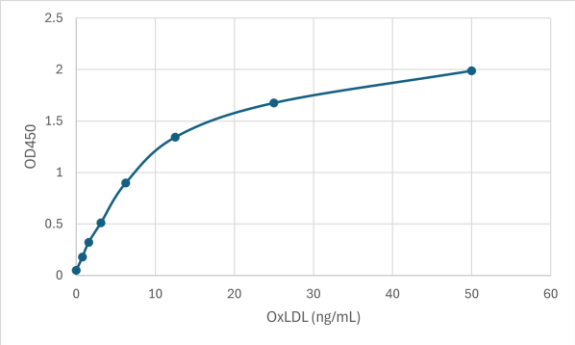


Figure 1. A 50 ng/mL OxLDL standard was serially diluted two-fold on an ELISA plate coated with anti-OxLDL capture antibody and quantified using a sandwich ELISA with an anti–OxLDL detection antibody.