Human Oxidized LDL (HNE-LDL) ELISA Kit

Human Oxidized LDL (HNE-LDL) ELISA Kit is an Enzyme Immunoassay kit for the quantification of Human Oxidized LDL (HNE-LDL) in Human serum, plasma.

Catalog number: ARG83383

Package: 96 wells

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INTRODUCTION

Oxidized LDL is a general term for LDL particles with oxidatively modified structural components. As a result, from free radical attack, both lipid and protein parts of LDL can be oxidized in the vascular wall. Besides the oxidative reactions taking place in vascular wall, oxidized lipids in LDL can also be derived from oxidized dietary lipids. Oxidized LDL is known to associate with the development of atherosclerosis, and it is therefore widely studied as a potential risk factor of cardiovascular diseases. Atherogenicity of oxidized LDL has been explained by lack of recognition of oxidation-modified LDL structures by the LDL receptors, preventing the normal metabolism of LDL particles and leading eventually to development of atherosclerotic plaques. Of the lipid material contained in LDL, various lipid oxidation products are known as the ultimate atherogenic species. Acting as a transporter of these injurious molecules is another mechanism by which LDL can increase the risk of atherosclerosis.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Human Oxidized LDL (HNE-LDL) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Human Oxidized LDL (HNE-LDL) present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Human Oxidized LDL (HNE-LDL) is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Human Oxidized LDL (HNE-LDL) bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ± 2nm. The concentration of Human Oxidized LDL (HNE-LDL) in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard and 100X Blocking Buffer at ≤ -20°C.

Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C
Standard	25 µL; 0.5 mg/mL	4°C
Diluent Buffer	50 mL	4°C
1000X Antibody	20 µL	-20°C
1000X HRP-Streptavidin Solution	20 µL	4°C
2X Precipitation Solution	20 mL	4°C.
10X Wash Buffer	100 mL	4°C
100X Blocking Buffer	200 µL	-20°C
TMB Substrate	12 mL	4°C (Protect from light)
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store Standard and 100X Blocking Buffer at ≤ -20°C.Store other component at 2-8°C at all times. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 10 minutes before centrifugation for 15 minutes at 1000 x g at 4°C. Remove 200 µL of serum and add 200 µL Precipitation Solution, mixing well. Incubate at room temperature for 5 minutes (precipitation will occur). Centrifuge for 20 minutes at 2000 x g (pellet should be visible). Carefully aspirate the supernatant and collect the pellet. Resuspend and dissolve the pellet in 1.6 mL of PBS, vortexing well. Further dilute the sample 1:50 to 1:200 in Assay Diluent before running the ELISA. Assay immediately and do not store solutions.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 10 minutes at 1000 x g. within 30 minutes at 4°C of collection. Remove 200 µL of plasma and add 200 µL Precipitation Solution, mixing well. Incubate at room temperature for 5 minutes (precipitation will occur). Centrifuge for 20 minutes at 2000 x g (pellet should be visible). Carefully aspirate the supernatant and collect the pellet. Resuspend and dissolve the pellet in 1.6 mL of PBS, vortex well. Further dilute the sample 1:50 to 1:200 in Assay Diluent before running the ELISA. Assay immediately and do not store solutions.

Note:

- Do not use haemolytic, icteric or lipaemic specimens.
- Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute **10X** Wash Buffer into distilled water to yield 1X Wash Buffer.
- **1X Antibody:** Immediately before use, dilute **1000X** Antibody into Diluent Buffer to yield 1X Antibody.
- **1X HRP-Streptavidin Solution:** Immediately before use, dilute **1000X** HRP-Streptavidin Solution into Diluent Buffer to yield 1X HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- **1X Blocking Buffer:** Immediately before use, dilute the **100X** Blocking Buffer into PBS to yield 1X Blocking Buffer.
- **Standard:** Centrifuge for spin down the material prior to open the vial. The Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer. Diluted the standard as below:

Standard tube	Human Oxidized LDL (HNE-LDL)(μg/mL)	Diluent Buffer (μL)	Standard (μL)
S1	1000	998	2(0.5 mg/mL Standard Stock)
S2	500	250	250 of S1
S3	250	250	250 of S2
S4	125	250	250 of S3
S5	62.5	250	250 of S4
S6	31.25	250	250 of S5
S7	15.6	250	250 of S6
S0	0	250	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

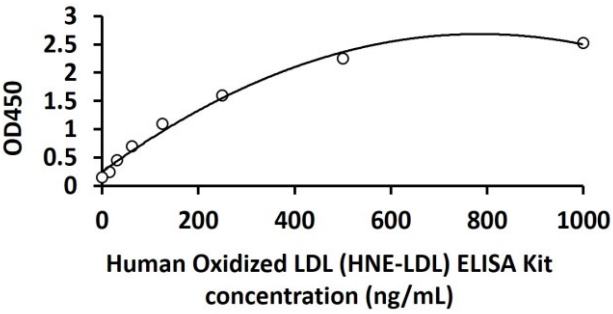
1. Add **100 µL** of **Precipitation and diluent Samples**, **Standard** into respective wells of the 96-well plate.
2. Cover the plate and incubate for **2 hour** at **room temperature**.
3. Aspirate each well and wash, repeating the process 2 time for a **total 3 washes.** Wash by filling each well with **1X Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X Blocking Buffer** to each well.
5. Cover the plate and incubate for **1 hour** at **room temperature**.
6. Aspirate each well and **wash** plate **as step 3**, but for **total 5 washes**
7. Add **100 µL** of **1X Antibody** to each well.
8. Cover the plate and incubate for **1 hour** at **room temperature**.
9. Aspirate each well and **wash** plate **as step 6**.
10. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
11. Cover the plate and incubate for **1 hour** at **room temperature** in the dark.
12. Aspirate each well and **wash** plate **as step 6**.
13. Add **100 µL** of **TMB Substrate** in each well.
14. Incubate for **5-30 mins** at **room temperature** in the dark.
15. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
16. Read the absorbance with a plate reader at **O.D. 450 nm** within 10 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Human Oxidized LDL (HNE-LDL) ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

15 ng/ml

Assay Range

15.63 - 1000 ng/mL