Human Oxidized LDL ELISA Kit

Cat. No.: DEIA1760
Pkg. Size: 96T

Intended use

The Human Oxidized LDL ELISA Kit is intended to be used for the quantitative measurement of oxidized low density lipoproteins (oxidized LDL) in human blood serum or plasma. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases.

General Description

The oxidative conversion of low density lipoproteins (LDL) to oxidized low density lipoproteins (oxidized LDL) is now considered to be a key event in the biological process that initiates and accelerates the development of the early atherosclerotic lesion, the fatty streak. Experimental studies have shown that native LDL becomes atherogenic when it is converted to oxidized LDL, and that oxidized LDL is more atherogenic than native LDL. Oxidized LDL is found in monocyte-derived macrophages in atherosclerotic lesions, but not in normal arteries. The uptake of LDL into macrophages does not occur by way of the classic Brown/Goldstein LDL receptor. Numerous studies have established that LDL, the major carrier of blood cholesterol, must first be converted to oxidized LDL so that it can be recognized by "scavenger" or "oxidized LDL" receptors on monocyte-derived macrophages. The binding of oxidized LDL to macrophages is a necessary step by which oxidized LDL induces cholesterol accumulation in macrophages, thus transforming the macrophages into lipid-laden foam cells. Holvoet and his colleagues were the first to clearly demonstrate that patients with coronary artery disease had significantly elevated plasma levels of oxidized LDL, and that these circulating levels of oxidized LDL were very similar in patients with stable coronary artery disease and in patients with acute coronary syndromes. They found plasma oxidized LDL results to be significantly higher in patients with stable angina, unstable angina and acute myocardial infarction when compared to age-matched, presumably healthy, control subjects. In the publication of Holvoet, plasma oxidized LDL levels were measured by a competitive ELISA utilizing a specific murine monoclonal antibody, mAb-4E6. It should be noted that the Oxidized LDL ELISA kit uses the same specific murine monoclonal antibody, mAb-4E6, that Holvoet used in his assays. However, the assay kit is a capture ELISA (also known as a "sandwich" ELISA), in which the wells of the microtiter plates are coated with the capture antibody, mAb-4E6. Several noteworthy studies have been reported by clinical researchers who have used the Oxidized LDL ELISA kits. Hulthe and Fagerberg demonstrated the relationship between subclinical atherosclerosis and circulating oxidized LDL levels by showing that oxidized LDL levels were related to intima-media thickness and plaque occurrence in the carotid and femoral arteries. Sigurdardottir, Fagerberg and Hulthe found elevated levels of oxidized LDL in patients with metabolic syndrome. In addition, they found that elevated oxidized LDL levels in metabolic syndrome patients were associated with small LDL-particle size. Kopprasch et al found elevated levels of circulating oxidized LDL in subjects with impaired glucose tolerance (IGT). And Duntas, Mantzou, and Koutras found significantly elevated plasma oxidized LDL levels in untreated patients with overt hypothyroidism. At the American Heart Association Scientific Sessions 2002, Johnston et al reported that plasma levels of oxidized LDL were substantially higher in patients with unstable coronary artery disease compared to healthy controls. Most important, there was no significant difference between the cholesterol levels of the unstable coronary artery disease patients and the healthy controls.

Principle Of The Test

Human Oxidized LDL ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. During incubation oxidized LDL in the sample reacts with anti-oxidized LDL antibodies bound to microtiter well. After washing, which removes non-reactive plasma components, a peroxidase conjugated anti-human apolipoprotein B antibody
recognizes the oxidized LDL bound to the solid phase. After a second incubation and a simple washing step that removes unbound enzyme labeled antibody, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint then read spectrophotometrically at 450 nm.

**Reagents And Materials Provided**

Each Oxidized LDL ELISA kit contains reagents for 96 wells, sufficient for 40 samples, two Controls and one calibration curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical Lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is ±2-8 °C.

1. Coated Plate 1 plate, 8-well strips 96 wells. Ready for use (mouse monoclonal anti-oxidized LDL). For unused microplate wells, completely reseal the bag using adhesive tape and use within two months.
2. Calibrators 5 vials 1000 μL Lyophilized. Concentrations stated on vial label (human oxidized LDL). Add 1000 μL redistilled water per vial.
3. Calibrator 0 vial 1000 μL Ready for use. Colour coded yellow.
4. Controls (L), (H) 2 vials 1000 μL Pre-diluted, lyophilized. Concentrations stated on vial label. Add 1000 μL redistilled water per vial.
5. Enzyme Conjugate 11X 1 vial 1.2 mL Preparation see below. (Peroxidase conjugated mouse monoclonal anti-apoB (6 μg/mL)). **Note:** Light sensitive.
7. Assay Buffer 1 vial 12 mL Ready for use.
8. Colour coded red.
9. Sample Buffer 4X 1 bottle 50 mL. Colour coded yellow. Dilute with 150 mL redistilled water to make Sample Buffer. **Note:** Precipitate may occur when stored at ±2-8 °C Allow Sample Buffer 4X to reach room temperature. Shake or vortex until precipitate has dissolved.
10. Wash Buffer 21X 1 bottle 40 mL. Dilute with 800 mL redistilled water to make Wash Buffer.
11. Substrate TMB 1vial 22 mL Ready for use. Colourless solution. **Note:** Light sensitive!
12. Stop Solution1 vial 7 mL Ready for use. 0.5 M H2SO4.

**Materials Required But Not Supplied**

1. 25 μL micropipette with disposable tips
2. 50 μL, 100 μL, 200 μL and 1000 μL repeating pipettes
3. Beakers and cylinders for reagent preparation
4. Redistilled water
5. Test tubes with caps, 3.5 mL
6. Microplate reader with 450 nm filter
7. Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
8. Wash device for microplates
9. "Vortex"-mixer

**Storage**

1. The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
2. The Stop Solution in this kit contains 0.5 M H2SO4. Follow routine precautions for handling hazardous chemicals.
3. All patient samples should be handled as capable of transmitting infections.

**Specimen Collection And Handling**

The recommended use of specimen in the Oxidized LDL ELISA is fresh EDTA-plasma. Heparin-plasma and serum may also be
used.

**Plasma:**
Collect blood by venipuncture into tubes containing EDTA or heparin as anticoagulant, and separate the plasma fraction.
Samples can be stored at -80 °C for at least six months. Avoid repeated freezing and thawing.

**Serum:**
Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Samples can be stored at -80 °C for at least six months. Avoid repeated freezing and thawing.

### Assay Steps

All reagents and samples must be brought to room temperature before use. Prepare a standard curve for each assay run.

1. Prepare sufficient Coated Plate wells to accommodate Calibrators, Controls and samples in duplicate
2. Pipette 25 μL of each Calibrator, Control and diluted sample into appropriate wells.
3. Add 100 μL Assay Buffer to each well.
4. Incubate on a plate shaker for 2 hours at room temperature (18-25 °C).
5. Wash 6 times with automatic washer or: Aspirate the reaction volume and fill each well completely with 350 μL Wash Buffer. Discard liquid completely. Repeat 5 times. After final wash, invert and tap the plate firmly against absorbent paper.
6. Add 100 μL Enzyme Conjugate Solution to each well.
7. Incubate on a plate shaker for 1 hour at room temperature (18-25 °C).
8. Wash as described above.
9. Add 200 μL Substrate TMB.
10. Incubate for 15 minutes at room temperature, no shaking.
11. Add 50 μL Stop Solution. Place plate on the shaker for 15 seconds to ensure mixing.
12. Read optical density at 450 nm and calculate results. Read within 30 minutes.

### Quality Control

It is good laboratory practice to record the following data for each assay: kit lot number; reconstitution dates of kit components; OD values for the blank, Calibrators and Controls.

### Calculation

**Computerized calculation:**
The concentration of oxidized LDL is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator O, versus the concentration using cubic spline regression. Multiply the concentration of the unknown samples with the dilution factor (e.g. x 6561).

**Manual calculation:**
1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the Oxidized LDL concentration on a lin-log paper and construct a calibration curve. 2. Read the concentration of the Controls and unknown samples from the calibration curve. Multiply the concentration of the Controls and the unknown samples with the dilution factor (e.g. x 6561).

### Reference Values

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own.

### Recovery

Recovery upon addition is 85-107% (mean value is 95%).
Reproducibility

Precision was calculated from three samples assayed in 3-8 replicates on 20 different occasions.

Limitations

1. As with all diagnostic tests, a definitive diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.
2. Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.