Mercodia Oxidized LDL ELISA

Directions for Use

REF 10-1143-03
Reagents for 96 determinations

For Research Use Only
Not for Use in Diagnostic Procedures

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31-3211
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Explanation of symbols used on labels

	Reagents for 96 determinations
	Use-by date
	Temperature limit
	Batch code
	Catalogue number
	Consult electronic Instructions for Use
	Manufacturer

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Intended use

The Mercodia Oxidized LDL ELISA kit is intended to be used for the *in vitro* quantitative measurement of oxidized low-density lipoproteins (oxidized LDL) in human serum or plasma.

Summary and explanation of the test

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[1–6].

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[1–6]. Oxidized LDL is found in monocyte-derived macrophages in atherosclerotic lesions[7]. The uptake of LDL into macrophages does not occur by way of the classic Brown/Goldstein LDL receptor[8]. 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[9–11].

Holvoet and 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 levels to be significantly higher in patients with stable angina, unstable angina and acute myocardial infarction when compared to age matched, presumably healthy control subjects.[12]

In publications by Holvoet et al., plasma oxidized LDL levels were measured by a competitive ELISA utilizing a specific murine monoclonal antibody mAb-4E6[12–16]. It should be noted that the Mercodia Oxidized LDL ELISA kit uses the same specific murine monoclonal antibody, mAb-4E6, that Holvoet et al. used in their assays. However, the Mercodia 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 Mercodia Oxidized LDL ELISA kits. As an example, 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[17]. Sigurdardottir, Fagerberg and Hulthe found elevated levels of oxidized LDL in patients with metabolic syndrome[18]. In addition, they found that elevated oxidized LDL levels in metabolic syndrome patients were associated with small LDL-particle size[18]. Kopprasch et al. found elevated levels of circulating oxidized LDL in subjects with impaired glucose tolerance (IGT)[19]. And Duntas, Mantzou and Koutras found significantly elevated plasma oxidized LDL levels in untreated patients with overt hypothyroidism[20].

Principle of the procedure

Mercodia 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 microtitration 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.

Warnings and precautions

- All samples should be handled as of capable of transmitting infections.
- Each well can only be used once.
- Do not mix components from different lots.
- Dispose of used components according to local regulations for biohazardous waste.

For safety information, please consult the safety data sheets (SDS) available at our website https://www.mercodia.com/technical-library/.

Materials required but not provided

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of Assay Buffer, enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagent preparation
- Test tubes with caps for sample dilution
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader with 450 nm filter
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

Reagents

Each Mercodia Oxidized LDL ELISA kit contains reagents for 96 wells, sufficient for 39 samples, two Controls and one calibrator 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.

Coated Plate Mouse monoclonal anti-oxidized LDL For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 1 month	1 plate	96 wells 8-well strips	Ready for Use
Calibrators 1, 2, 3, 4, 5, 6 Human oxidized LDL Color coded yellow Concentration indicated on vial label Storage after reconstitution: 2–8°C for 1 month	6 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial. Ensure mixing after reconstitution.
Calibrator 0 Color coded yellow *Note! Negative control!*	1 vial	1000 µL	Ready for Use
Controls (L), (H) Antigen concentration indicated on vial label Storage after reconstitution: 2–8°C for 1 month	2 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial. Ensure mixing after reconstitution.
Enzyme Conjugate 15X Peroxidase conjugated mouse monoclonal anti-apoB	1 vial	1.8 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	25 mL	Ready for Use
Assay Buffer Color coded red	1 vial	22 mL	Ready for Use
Sample Buffer 4X Color coded yellow Storage after dilution: 2–8 °C for 1 month	1 bottle	50 mL	Preparation, see Dilution of Samples below
Wash Buffer 21X Storage after dilution: 2–8°C for 2 months	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution
Substrate TMB Colorless solution *Note! Light sensitive!*	1 bottle	22 mL	Ready for Use
Stop Solution 0.5 M H_2SO_4	1 vial	7 mL	Ready for Use

Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 15X (1+14) in Enzyme Conjugate Buffer according to the table below. When preparing enzyme conjugate 1X solution for the whole plate, pour all the Enzyme Conjugate Buffer into the Enzyme Conjugate 15X vial. Mix gently. Store at 2–8°C and use within 1 month.

Number of strips	Enzyme Conjugate 15X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	1000 µL	14.0 mL
4 strips	500 µL	7.0 mL

Specimen collection and handling

The recommended use of specimen in the Mercodia Oxidized LDL ELISA is EDTA-plasma or serum. Heparin-plasma may also be used. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Plasma

Collect blood by venipuncture in tubes containing EDTA or heparin as anticoagulant, and separate the plasma fraction by centrifugation. Avoid long time storage of samples at room temperature or 2–8°C. Oxidized LDL in EDTA plasma samples was found to be stable one hour at room temperature and 14 days at 2–8°C. Samples can be stored at –80°C for at least 6 months. Avoid repeated freezing and thawing.

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Avoid long time storage of samples at room temperature or 2–8°C. Oxidized LDL in serum samples was found to be stable one hour at room temperature and 14 days at 2–8°C. Samples can be stored at –80°C for at least 6 months. Avoid repeated freezing and thawing.

Dilution of samples

The dilution of samples is a very important step in the assay procedure*. If the dilution is not properly performed there is a risk of increased variation in measured oxidized LDL concentration. Each sample must be diluted in two steps for a final dilution of 1/6561. Samples may be thawed in room temperature or on ice. Avoid keeping undiluted samples in room temperature for more than one hour.

1. Prepare sample buffer 1X solution by diluting one bottle Sample Buffer 4X** (50 mL) in 150 mL redistilled water. Mix approximately 15 minutes using a magnetic stirrer to ensure a homogenous solution.
2. Add 10 µL of each sample to individual tubes.
3. Add 800 µL sample buffer 1X solution to each tube for a 1/81 dilution.
4. Cap all tubes of the first dilution and mix thoroughly using a vortex mixer and by inverting the tubes.
5. Add 10 µL of each 1/81 dilution to new individual tubes.
6. Add 800 µL sample buffer 1X solution to each tube for a final dilution of 1/6561.
7. Cap all tubes of the second dilution and mix thoroughly using a vortex mixer and by inverting the tubes.
8. Let each final sample dilution sit on the bench for 10 minutes and then mix again before the samples are added to the plate. The assay should be started within one hour of dilution and the diluted samples should not be stored.

*Note: The included controls (L) and (H) are ready to be used in the ELISA without this dilution procedure.

**Note: A precipitate may form in the Sample Buffer 4X when stored at 2–8°C. Allow the buffer to reach room temperature and mix until the precipitate has dissolved before diluting the concentrate in redistilled water.

Test procedure

All reagents and samples must be brought to room temperature before use. Prepare a standard curve for each assay run. The product has been optimized and validated without plate sealer.

1. Prepare sample buffer 1X solution and dilute samples according to instructions for both processes in the Dilutions of Samples section.
2. Prepare Calibrators, Controls, enzyme conjugate 1X solution and wash buffer 1X solution according to the Reagents section.
3. Prepare sufficient microplate wells to accommodate Calibrators 1-6, Calibrator 0 (negative control), positive controls, and samples in duplicate.
4. Add 200 µL Assay Buffer to each well.
5. Pipette 25 µL of each Calibrator, Control and diluted sample into appropriate wells. Once started, Calibrators, Controls and diluted samples should be added to the plate within 20 minutes.
6. Incubate on a plate shaker (700–900 rpm) for 2 hours at room temperature (18–25°C).
7. Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function. The chosen program should fill all the wells with wash buffer in each cycle and ensure that the wells are never left without wash buffer (e.g. Plate Mode). Do not use additional soak. Invert and tap the plate firmly against absorbent paper after the final wash.
   Or manually:
   Discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash buffer 1X solution to each well. Discard the wash solution, tap firmly several times against absorbent paper to remove excess liquid.
   Repeat 5 times. Avoid prolonged soaking during washing procedure.
8. Add 200 µL enzyme conjugate 1X solution to each well.
9. Incubate on a plate shaker (700–900 rpm) for 1 hour at room temperature (18–25°C).
10. Wash as described in 7.
11. Add 200 µL Substrate TMB.
12. Incubate on the bench for 15 minutes at room temperature, no shaking.
13. Add 50 µL Stop Solution. Place plate on the shaker for 5 seconds to ensure mixing.
14. Read optical density at 450 nm and calculate results.
    Read within 30 minutes.

Note! Be extra careful not to contaminate the Substrate TMB with enzyme conjugate solution.

Internal quality controls

Controls included in the kit and/or internal plasma/serum pools with low, intermediate and high oxidized LDL concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, preparation date of components, OD values for Calibrators 1-6, and the positive and negative controls.

Calculation of results

The concentration of Oxidized LDL is determined by plotting the optical density (OD) at 450 nm of Calibrators 1-6 against their respective concentrations. Calibrator 0 should be used solely as a negative control and not included in the calibration curve. It is important to use an appropriate curve fitting model that represents the true dose-response relationship to get accurate results. The Mercodia Oxidized LDL ELISA is validated using Magellan software (Tecan) with Five Parameter Logistic (5PL) and automatic weighting using $1/Y^2$.

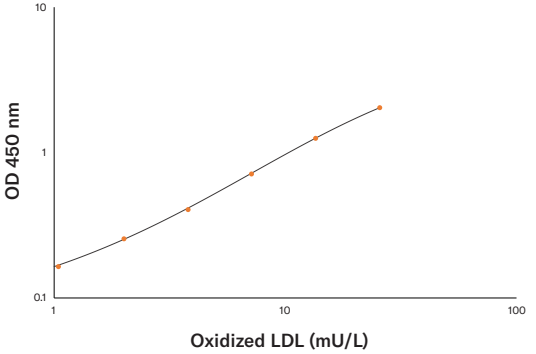
Example of results

Wells	Identity	$A_{450\ nm}$	Conc. mU/L	Conc. U/L**
1 A–B	Calibrator 0	0.075		
1 C–D	Calibrator 1*	0.165		
1 E–F	Calibrator 2*	0.256		
1 G–H	Calibrator 3*	0.407		
2 A–B	Calibrator 4*	0.715		
2 C–D	Calibrator 5*	1.252		
2 E–F	Calibrator 6*	2.036		
2 G–H	Sample 1	0.729	7.32	48.0
3 A–B	Sample 2	1.142	12.1	79.4
3 C–D	Sample 3	1.520	17.0	112

*Concentration stated on vial label.
**Results multiplied by dilution factor (× 6561), for comparison with concentration range of samples.

Example of calibrator curve

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.

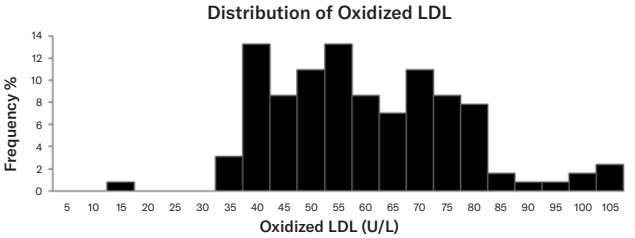


Limitations of the procedure

Grossly lipemic or hemolyzed samples do not interfere in the assay.

Expected values

Good practice dictates that each laboratory establishes its own expected range of values. The following results and graph may serve as a guide until the laboratory has gathered sufficient data of its own. Levels for 128 probes tested, in apparently healthy individuals, yielded a mean of 57.8 U/L, a median of 55.0 U/L and a central 95% reference range of 32.2–101 U/L analyzed in EDTA-plasma.



Performance characteristics

The assay is validated with a fit for purpose approach. Selected studies are presented here. Additional data can be obtained from Mercodia AB.

Validation of Curve fit

The curve fitting was validated with Five Parameter Logistics (weighted fit $1/Y^2$).

Detection limit

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured. The detection limit is 0.5 mU/L as determined by the methodology described in ISO11843-Part 4[21].

Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Mean recovery for dilutional linearity is 93% (82%–107%).

High Dose Hook Effect

Samples with a concentration up to 20X Cal 6 can be measured without giving falsely low results.

Precision

Six samples (EDTA plasma and serum) were analyzed in two replicates over 12 different occasions on one kitlot by four laboratory technicians.

		Coefficient of variation	
Sample	Mean value U/L	Repeatability %*	Within laboratory %**
EDTA plasma 1	41.6	2.8	7.2
EDTA plasma 2	45.8	2.1	8.8
EDTA plasma 3	65.4	3.3	9.8
Serum 1	47.4	2.3	7.8
Serum 2	62.6	1.7	9.7
Serum 3	49.9	2.7	8.9

*Within assay variation
**Total assay variation

Calibration

No international reference is at date available. The Mercodia Oxidized LDL ELISA is calibrated in relative arbitrary units against an in house reference preparation.

Warranty

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

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Further references can be found on our website: **www.mercodia.com**

Summary of protocol sheet
Mercodia Oxidized LDL ELISA

Add Assay Buffer to all wells	200 µL
Add Calibrators, Controls and diluted samples within 20 minutes	25 µL
Incubate	2 hrs at 18–25°C on a plate shaker (700–900 rpm)
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add enzyme conjugate 1X solution to all wells	200 µL
Incubate	1 h at 18–25°C on a plateshaker (700–900 rpm)
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add Substrate TMB	200 µL
Incubate	15 minutes at 18–25°C on the bench
Add Stop Solution	50 µL Shake for 5 sec to ensure mixing
Measure $A_{450\ nm}$	Read within 30 minutes

For full details see page 8.

For technical support please contact: support@mercodia.com

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