

# Merckodia Oxidized LDL Competitive ELISA

Directions for Use

**10-1158-01**





**REAGENTS FOR 96 DETERMINATIONS**

For Research Use only  
Not for Use In Diagnostic Procedures

Manufactured by  
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Merckodia 

## EXPLANATION OF SYMBOLS USED ON LABELS

|  |                                |
|--|--------------------------------|
| <br>$\Sigma = 96$ | Reagents for 96 determinations |
|                   | Expiry date                    |
|                   | Store between 2–8°C            |
|                   | Lot no                         |

## INTENDED USE

The Mercodia Oxidized LDL Competitive Enzyme-Linked Immunosorbent Assay (ELISA) kit is intended to be used for the in vitro quantitative determination of oxidized low density lipoproteins (oxidized LDL) in human blood plasma. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases.

## 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 [1–5].

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 [1–5]. Oxidized LDL is found in monocyte-derived macrophages in atherosclerotic lesions, but not in normal arteries [6]. The uptake of LDL into macrophages does not occur by way of the classic Brown/Goldstein LDL receptor [7]. Numerous studies [1–5,8] 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, and thus transforms the macrophages into lipid-laden foam cells [8].

Holvoet and his colleague’s [9] 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 [9,10], plasma oxidized LDL levels were measured by a competitive ELISA utilizing a specific murine monoclonal antibody, mAb-4E6. The Mercodia Oxidized LDL Competitive ELISA kit utilize the same assay technology and the same specific murine monoclonal antibody, mAb-4E6, that Holvoet used in his assay [9,10,11,12,13].

Several noteworthy studies have been reported by clinical researchers who have used the Mercodia Oxidized LDL ELISA kits. Hulthe and Fagerberg [14] 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 [15] 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 [16] found elevated levels of circulating oxidized LDL in subjects with impaired glucose tolerance (IGT). And Duntas, Mantzou, and Koutras [17] found significantly elevated plasma oxidized LDL levels in untreated patients with overt hypothyroidism.

At the American Heart Association Scientific Sessions 2002, Johnston et al [18] 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 PROCEDURE

Mercodia Oxidized LDL Competitive ELISA is based on the monoclonal antibody 4E6. Oxidized LDL in the sample competes with a fixed amount of oxidized LDL bound to the microtiter well for the binding of the biotin- labelled specific antibodies. After a washing step that removes unreactive sample components, the biotin-labelled antibody bound to the well is detected by HRP-conjugated streptavidin. After a second incubation and an additional washing step, 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 that is read spectrophotometrically.

## WARNINGS AND PRECAUTIONS

- For Research Use only. Not for Use in Diagnostic Procedures. Not for internal or external use in humans or animals.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop Solution in this kit contains 0.5 M H<sub>2</sub>SO<sub>4</sub>. Follow routine precautions for handling hazardous chemicals.
- All patient samples should be handled as capable of transmitting infections.

**Warning!** This kit contains reagents that may be infectious!

This kit contains reagents manufactured from human blood components. The source of material have been tested by immunoassay for hepatitis B surface antigen and for antibodies for HIV virus and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/national guidelines on laboratory safety procedures.

## SPECIMEN COLLECTION AND HANDLING

The recommended use of specimen in the Mercodia Oxidized LDL Competitive ELISA is fresh EDTA-plasma.

Collect blood by venipuncture into tubes containing EDTA as anticoagulant and separate the plasma fraction. Samples can be stored at 2–8°C up to one week or at –80°C for at least six months. Avoid repeated freezing and thawing.

Serum and heparin-plasma may also be used.

## **MATERIAL REQUIRED BUT NOT PROVIDED**

- 25  $\mu$ l micropipette with disposable tips
- 50  $\mu$ l, 100  $\mu$ l, 200  $\mu$ l and 1000  $\mu$ l repeating pipettes
- 1000 ml beaker
- Redistilled water
- Test tubes, 3.5 ml
- EIA plate reader with 450 nm filter
- Plate shaker
- Wash device for microtitration plates
- "Vortex"-mixer

## REAGENTS

Each Mercodia Oxidized LDL Competitive ELISA kit contains reagents for 96 wells, sufficient for 41 samples and one standard 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.

|   |          |          |  |
|---|----------|----------|--|
| <b>Coated Plate</b><br>(human oxidized LDL)   | 1 plate  | 96 wells | Ready for use<br>8-wells strips  |
| <b>Calibrators</b><br>(human oxidized LDL)<br>Conc. indicated at vial label<br>Color coded yellow | 5 vials  | 500 µl   | Lyophilized<br><br>Add 500 µl redistilled<br>water per vial                                      |
| <b>Calibrator 0</b><br>Colour coded yellow  | 1 vial   | 2.0 ml   | Ready for use  |
| <b>Enzyme Conjugate 11X</b><br>(Streptavidin-HRP)   | 1 vial   | 1.2 ml   | Preparation, see below   |
| <b>Enzyme Conjugate Buffer</b><br>Colour coded blue   | 1 vial   | 12 ml    | Ready for use  |
| <b>Antibody 6X</b><br>(mouse monoclonal anti-oxidized LDL)  | 1 vial   | 1000 µl  | Lyophilized<br>Add 6ml Antibody Buffer<br>to make Antibody                                       |
| <b>Antibody Buffer</b><br>Color coded red   | 1 vial   | 6 ml     | Ready for use  |
| <b>Sample Buffer</b><br>Color coded yellow  | 1 bottle | 50 ml    | Ready for use  |
| <b>Wash Buffer 21X</b>  | 1 bottle | 40 ml    | Dilute 1:21 add 1 part<br>concentrate to 20 parts of<br>redistilled water to make<br>Wash Buffer |
| <b>Substrate TMB</b><br><i>Note! Light sensitive!</i>   | 1 vial   | 22 ml    | Ready for use  |
| <b>Stop Solution</b><br>0.5 M H <sub>2</sub> SO <sub>4</sub>                                      | 1 vial   | 7 ml     | Ready for use  |

## Preparation of Enzyme Conjugate

Dilute the Enzyme Conjugate 11X (1.2ml) by adding the Enzyme Conjugate Buffer (12ml).

If less than 12 strips are used prepare the needed volume of Conjugate solution by mixing 100  $\mu$ l Enzyme Conjugate 11X with 1000  $\mu$ l Enzyme Conjugate Buffer (1+10) for each strip, as indicated table below:

| Number of strips | Enzyme Conjugate 11X | Enzyme Conjugate buffer |
|------------------|----------------------|-------------------------|
| 2 strips         | 200 $\mu$ l          | 2.0 ml                  |
| 4 strips         | 350 $\mu$ l          | 3.5 ml                  |
| 6 strips         | 600 $\mu$ l          | 6.0 ml                  |
| 8 strips         | 700 $\mu$ l          | 7.0 ml                  |
| 10 strips        | 900 $\mu$ l          | 9.0 ml                  |

Enzyme Conjugate preparation is stable for 4 weeks at +2–8°C following preparation.

## Dilution of samples

Samples must be diluted the same day as the assay performance. Prepare a tube for each patient sample. Each sample is diluted in one step to a final dilution of 41 times as follows:

|                |              |
|----------------|--------------|
| Patient sample | 25 $\mu$ l   |
| Sample Buffer  | 1000 $\mu$ l |

It is IMPORTANT to ensure that the samples are properly mixed before further use.

Sample diluted 41 times in Sample Buffer is stable for 1 day at +4°C.

## Stability of reconstituted and opened reagents

|   |                  |
|---|------------------|
| Calibrators                               | 2 weeks at 2–8°C |
| Antibody Solution                         | 2 weeks at 2–8°C |
| Enzyme Conjugate                          | 2 weeks at 2–8°C |
| Sample Buffer                             | 2 weeks at 2–8°C |
| Wash Buffer                               | 4 weeks at 2–8°C |
| Coated plate, unused strips, resealed bag | 2 weeks at 2–8°C |

## TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. Perform each determination in duplicate for B<sub>0</sub>, Blank, Calibrators, Controls and Unknowns. Prepare a Calibration curve for each assay run.

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| Add to Oxidized LDL wells: | B <sub>0</sub> | Blank | Calibrators | Unknown |
|----------------------------|----------------|-------|-------------|---------|
| 1 Calibrators 0            | 50 µl          | 100µl | –           | –       |
| 2 Calibrators              | –              | –     | 50 µl       | –       |
| 3 Unknown                  | –              | –     | –           | 50 µl   |
| 4 Antibody                 | 50 µl          | –     | 50 µl       | 50 µl   |

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Please observe that mAb solution is added to all wells except to the Blank!

5 Incubate on a shaker for 2 hours at room temperature (18–25°C)

6 Wash 6 times with automatic washer, or manually by hand.

Wash 6 times with 350 µl Wash Buffer. Aspirate completely between each washing step.

After final wash, invert and tap the plate firmly against absorbent paper.

7 Add Enzyme Conjugate 100 µl to all wells

8 Incubate on a shaker for 1 hour at room temperature (18–25°C)

9 Wash 6 times with automatic washer, or manually by hand.

Wash 6 times with 350 µl Wash Buffer. Aspirate completely between each washing step.

After final wash, invert and tap the plate firmly against absorbent paper.

10 Add substrate TMB 200 µl to all wells

11 Incubate for 15 minutes at room temperature, no shaking.

12 Add Stop Solution 50 µl to all wells

Place the plate on a shaker for 5 seconds to ensure mixing.

13 Measure the absorbance at 450 nm and calculate the results.

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## INTERNAL QUALITY CONTROL

Commercial controls such as Mercodia oxidized LDL Control Kit (10-1165-01/10-1171-01) and/or internal plasma/serum pools with low, intermediate and high oxidized LDL concentrations should routinely be assayed as unknowns, and results charted from day to day. 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 B<sub>0</sub>, Blank, Calibrators and Controls.

## CALCULATION OF RESULTS

### Manual calculation

1. Express the absorbance (B) for Calibrators, Controls and Unknowns as a percentage inhibition of the mean absorbance of the maximum binding at zero inhibition ( $B_0$ )

$$\%B_0 = \frac{B \text{ (of Calibrators, Controls or Unknowns)}}{B_0 \text{ (mean abs at zero inhibition)}} \times 100$$

2. Plot the calculated percentage values ( $\%B_0$ ) obtained for the Calibrators against the log concentration on lin-log paper and construct a calibration curve.
3. Read the concentration of the Controls and Unknown samples from the calibration curve.
4. Multiply the concentration of the Controls and the Unknown samples with the dilution factor (eg.  $\times 41$ ).

### Computerized calculation

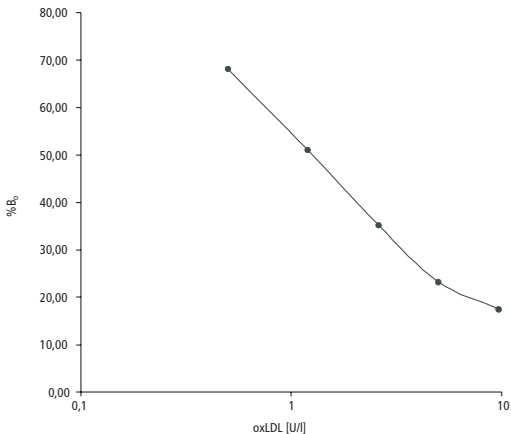
Computerised data reduction of " $\%B_0$ " for the calibrators versus the log (concentration) using a cubic spline regression algorithm may be performed to obtain the concentration of oxidized LDL. Multiply the concentration of the Controls and Unknown samples with the dilution factor (eg.  $\times 41$ ).

### Example of worksheet

| Wells | Identity   | Mean<br>$A_{450\text{nm}}$ | Mean<br>$\%B_0$ | Mean<br>Conc. U/l | $\times 41$<br>U/l |
|-------|------------|----------------------------|-----------------|-------------------|--------------------|
| 1A-B  | $B_0$      | 2.104                      |                 |                   |                    |
| 1C-D  | Blank      | 0.121                      | 5.7             |                   |                    |
|       | Calibrator |                            |                 |                   |                    |
| 1E-F  | 0.5 U/l    | 1.433                      | 68.1            |                   |                    |
| 1G-H  | 1.2 U/l    | 1.074                      | 51.0            |                   |                    |
| 2A-B  | 2.6 U/l    | 0.740                      | 35.2            |                   |                    |
| 2C-D  | 5.0 U/l    | 0.488                      | 23.2            |                   |                    |
| 2E-F  | 9.7 U/l    | 0.367                      | 17.4            |                   |                    |
|       | Controls   |                            |                 |                   |                    |
| 2G-H  | Control A  | 1.185                      | 56.3            | 0.9               | 37                 |
| 3A-B  | Control B  | 0.844                      | 40.1            | 2.0               | 82                 |
| 3C-D  | Control C  | 0.670                      | 31.7            | 3.1               | 127                |
|       | Unknown    |                            |                 |                   |                    |
| 3C-D  | Sample 1   | 1.211                      | 57.6            | 0.9               | 37                 |
| 3E-F  | Sample 2   | 0.914                      | 43.4            | 1.7               | 70                 |
| 3G-H  | Sample 3   | 0.758                      | 36.0            | 2.5               | 103                |

## EXAMPLE OF CALIBRATION CURVE

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



## PERFORMANCE CHARACTERISTICS

### Detection Limit

The detection Limit is  $\leq 0.3$  U/l.

### Recovery

Recovery upon addition is 93–115% (mean value is 101%)

## Precision

Precision was calculated from three samples assayed in 2–4 replicates on 21 different occasions.

| Sample | Obtained value<br>U/L | Coefficient of variation % |         |       |
|--------|-----------------------|----------------------------|---------|-------|
|        |                       | within                     | between | total |
| 1      | 39                    | 6.7                        | 6.9     | 9.6   |
| 2      | 82                    | 4.8                        | 4.5     | 6.5   |
| 3      | 128                   | 6.1                        | 7.0     | 9.3   |

## Dilutions

Recovery upon dilution for samples 1:21–1:42–1:84 is 1.01, range 0.85–1.16.

## Interference

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

## Calibration

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

## LIMITATIONS OF THE PROCEDURE

The product is only provided for research use and not for use in diagnostic procedures. As with all diagnostic tests, a definite 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.

## 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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## SUMMARY PROTOCOL SHEET

### Merckodia Oxidized LDL Competitive ELISA

|   |   |
|---|---|
| Add Calibrator 0 for B <sub>0</sub>               | 50 µl   |
| Add Calibrator 0 for Blank                        | 100 µl  |
| Add Calibrators, Controls and Samples             | 50 µl   |
| Add Antibody to all wells <u>except</u> the Blank | 50 µl   |
| Incubate  | 2 hours at 18–25°C on a plate shaker          |
| Wash plate with Washing Buffer                    | 6 times                                       |
| Add Enzyme Conjugate to all wells                 | 100 µl  |
| Incubate  | 1 hour at 18–25°C on a plate shaker           |
| Wash plate with Washing Buffer                    | 6 times                                       |
| Add Substrate TMB                                 | 200 µl  |
| Incubate  | 15 minutes                                    |
| Add Stop Solution                                 | 50 µl<br>Shake for 5 seconds to ensure mixing |
| Measure A <sub>450</sub>                          | Evaluate results                              |