

Mercodia

Oxidized LDL ELISA

Directions for Use

10-1143-01 REAGENTS FOR 96 DETERMINATIONS

For *in vitro* diagnostic use



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Veuillez trouver le mode d'emploi en français à:
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Manufactured by

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Sweden

EXPLANATION OF SYMBOLS USED ON LABELS

| | |
|--|------------------------------------|
|  $\Sigma = 96$ | Reagents for 96 determinations |
|  | Expiry date |
|  | Store between 2–8°C |
|  | Lot No. |
|  | For <i>in vitro</i> diagnostic use |

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 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.

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, thus transforming the macrophages into lipid-laden foam cells [8].

Holvoet and his colleagues [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 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.

In publications by Holvoet [9-13], plasma oxidized LDL levels were measured by a competitive ELISA utilizing a specific murine monoclonal antibody mAb-4E6. It should be noted that the Mercodia Oxidized LDL ELISA kit uses the same specific murine monoclonal antibody, mAb-4E6, that Holvoet [9,10] used in his 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. 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. (References page 13)

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

- For *in vitro* diagnostic use. 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 H₂SO₄. 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, antibodies for Hepatitis C virus 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.

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes for 25 µL, 50 µL, 100 µL, 200 µL and 1000 µL (repeat pipettes preferred for addition of enzyme 1X conjugate solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water
- Test tubes with caps, 3.5 mL
- Microplate reader 450 nm filter
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device
- Appropriate mixing device

REAGENTS

Each Mercodia Oxidized LDL ELISA kit contains reagents for 96 wells, sufficient for 40 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 8 weeks. | 1 plate | 96 wells 8-well strips | Ready for Use |
| Calibrators 1, 2, 3, 4, 5 Human oxidized LDL Color coded yellow Concentration indicated on vial label. Storage after reconstitution: 2–8°C for 1 week. For storage of reconstituted Calibrators for more than 1 week, store at -20°C. | 5 vials | 1000 µL | Lyophilized Add 1000 µL redistilled water per vial. |
| Calibrator 0 Color coded yellow | 1 vial | 1000 µL | Ready for Use |
| Controls (H), (L) Antigen concentration indicated on vial label. Storage after reconstitution: 2–8°C for 1 week. For storage of reconstituted Controls for more than 1 week, store at -20°C. | 2 vials | 1000 µL | Lyophilized Add 1000 µL redistilled water per vial. |
| Enzyme Conjugate 11X Peroxidase conjugated mouse monoclonal anti-apoB | 1 vial | 1.2 mL | Preparation, see below |
| Enzyme Conjugate Buffer Color coded blue | 1 vial | 12 mL | Ready for use |
| Assay Buffer Color coded red | 1 vial | 12 mL | Ready for use |
| Sample Buffer 4X Color coded yellow <i>Note!</i> Precipitate may occur when stored at 2-8 °C. Allow Sample Buffer 4X to reach room temperature. Mix until precipitate has dissolved. Storage after dilution: 2-8 °C for 4 weeks. | 1 bottle | 50 mL | Dilute with 150 mL redistilled water to make sample buffer 1X solution. |
| Wash Buffer 21X Storage after dilution: 2-8°C for 8 weeks. | 1 bottle | 50 mL | Dilute with 1000 mL redistilled water to make wash buffer 1X solution |
| Substrate TMB Colorless solution <i>Note! Light sensitive!</i> | 1 bottle | 22 mL | Ready for Use |
| Stop Solution 0.5 M H ₂ SO 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 11X (1+10) in Enzyme Conjugate Buffer according to the table below. When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently.

| Number of strips | Enzyme Conjugate 11X | Enzyme Conjugate Buffer |
|------------------|----------------------|-------------------------|
| 12 strips | 1 vial | 1 vial |
| 8 strips | 700 μ L | 7.0 mL |
| 6 strips | 500 μ L | 5.0 mL |
| 4 strips | 350 μ L | 3.5 mL |

Storage after dilution: 2-8°C for 1 month.

SPECIMEN COLLECTION AND HANDLING

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

Plasma

Collect blood by venipuncture in 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.

DILUTION OF SAMPLES

Prepare two tubes for each patient sample. Each sample has to be diluted in two steps to a final dilution of 1/6561 as follows:

| | | | |
|---|---|----------------------------|-----------------|
| 1 | Patient samples Sample buffer 1X solution Cap tubes, invert three times and vortex-mix* | 25 μ L 2000 μ L | Dilution 1/81 |
| 2 | 1/81 dilution of sample Sample buffer 1X solution Cap tubes, invert three times and vortex-mix* | 25 μ L 2000 μ L | Dilution 1/6561 |

* It is IMPORTANT to ensure that each dilution step is properly mixed before the next step.

As a result of this procedure the samples will be diluted 1/6561.

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use.

Prepare a standard curve for each assay run.

1. Prepare enzyme conjugate 1X solution, sample buffer 1X solution, wash buffer 1X solution and samples.
2. Prepare sufficient Coated Plate wells to accommodate Calibrators, Controls and samples in duplicate.
3. Pipette 25 μ L of each Calibrator, Control and diluted sample into appropriate wells.
4. Add 100 μ L Assay Buffer to each well.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours at room temperature (18–25°C).
6. Wash 6 times with 700 μ L wash buffer 1X solution per well using an automatic plate washer with overflow-wash function. After final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure.

Or manually:

Discard the reaction volume by inverting the microplate over a sink. Add 350 μ L 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.

7. Add 100 μ L enzyme conjugate 1X solution to each well.
8. Incubate on a plate shaker for 1 hour at room temperature (18–25°C).
9. Wash as described in 6.
10. Add 200 μ L Substrate TMB.
11. Incubate for 15 minutes at room temperature, no shaking.
12. Add 50 μ L Stop Solution. Place plate on the shaker for 5 seconds to ensure mixing.
13. Read optical density at 450 nm and calculate results.
Read within 30 minutes.

Note! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

INTERNAL QUALITY CONTROL

Commercial controls 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, dilution and/or reconstitution dates of kit components OD values for the blank, Calibrators and Controls.

CALCULATION OF RESULTS

Computerized calculation

The concentration of oxidized LDL is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the concentration using cubic spline regression. Multiply the concentration of the samples with the dilution factor (e.g. $\times 6561$).

Manual calculation

1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the Oxidized LDL concentration on a log-log paper and construct a calibrator curve.
2. Read the concentration of the Controls and unknown samples from the calibrator curve.
3. Multiply the concentration of the Controls and the samples with the dilution factor (e.g. $\times 6561$).

Example of results

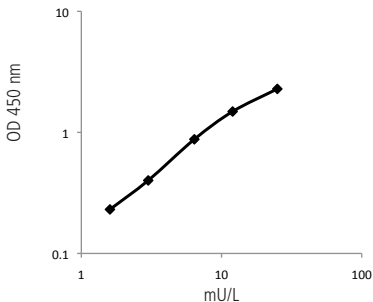
| Wells | Identity | A ₄₅₀ nm | Conc. mU/L | U/L** |
|-------|---------------|---------------------|------------|-------|
| 1A–B | Calibrator 0 | 0.072 | | |
| 1C–D | Calibrator 1* | 0.185 | | |
| 1E–F | Calibrator 2* | 0.369 | | |
| 1G–H | Calibrator 3* | 0.664 | | |
| 2A–B | Calibrator 4* | 1.405 | | |
| 2C–D | Calibrator 5* | 2.469 | | |
| 2E–F | Control (H) | 1.234 | 12.2 | 80.04 |
| 2G–H | Control (L) | 0.513 | 5.2 | 34.12 |

*Concentration stated on vial label.

**Result multiplied by dilution factor ($\times 6561$).

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

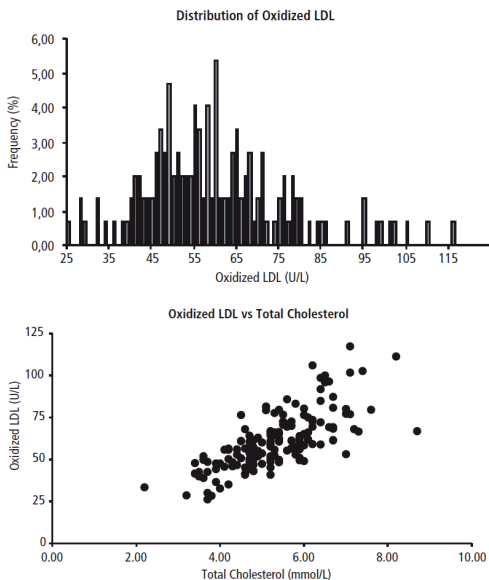
As with all diagnostic tests, a definitive diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.

Grossly lipemic, icteric 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 may serve as a guide until the laboratory has gathered sufficient data of its own.

The following results were obtained from 149 ambulatory, randomly selected individuals in the Stockholm area, Sweden.



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| | Mean | Median | Range |
|--------------------|------|--------|-----------|
| Range OxLDL (U/L)* | 61 | 59 | 26–117 |
| Chol/HDL ratio** | 4.10 | 3.90 | 1.68–7.89 |

* Arbitrary units. See CALIBRATION.

**Measured data Cholesterol (mmol/L) and HDL (mmol/L).

Distribution of Oxidized LDL and Cholesterol/HDL ratio.

| Oxidized LDL U/L | Cholesterol/HDL Ratio | Patient/Total (%) |
|---------------------|--------------------------|-------------------|
| Quartile 1 | | |
| Q1(26–49) | 1.68–3.13 | 22/149 (14.8) |
| Q2(50–59) | 1.68–3.13 | 10/149 (6.7) |
| Q3(60–69) | 1.68–3.13 | 5/149 (3.4) |
| Q4(70–117) | 1.68–3.13 | 0/149 (0.0) |
| Quartile 2 | | |
| Q1(26–49) | 3.21–3.86 | 7/149 (4.7) |
| Q2(50–59) | 3.21–3.86 | 17/149 (11.4) |
| Q3(60–69) | 3.21–3.86 | 10/149 (6.7) |
| Q4(70–117) | 3.21–3.86 | 3/149 (2.0) |
| Quartile 3 | | |
| Q1(26–49) | 3.87–4.79 | 7/149 (4.7) |
| Q2(50–59) | 3.87–4.79 | 11/149 (7.4) |
| Q3(60–69) | 3.87–4.79 | 11/149 (7.4) |
| Q4(70–117) | 3.87–4.79 | 9/149 (6.0) |
| Quartile 4 | | |
| Q1(26–49) | 4.80–7.89 | 1/149 (0.7) |
| Q2(50–59) | 4.80–7.89 | 4/149 (2.7) |
| Q3(60–69) | 4.80–7.89 | 7/149 (4.7) |
| Q4(70–117) | 4.80–7.89 | 25/149 (16.8) |

The following results were obtained from 147 ambulatory, randomly selected individuals in the Stockholm area, Sweden.

| | Mean | Median | Range |
|--------------------|------|--------|-----------|
| Range OxLDL (U/L)* | 61 | 59 | 26–117 |
| LDL/HDL ratio** | 2.51 | 2.36 | 0.55–5.56 |

* Arbitrary units. See CALIBRATION.

** Measured data LDL (mmol/L) and HDL (mmol/L).

Distribution of Oxidized LDL and LDL/HDL ratio.

| Oxidized LDL U/L | LDL/HDL Ratio | Patient/Total (%) | |
|---------------------|------------------|-------------------|--------|
| Quartile 1 | | | |
| Q1(26–49) | 0.55–1.79 | 19/147 | (13.0) |
| Q2(50–59) | 0.55–1.79 | 12/147 | (8.2) |
| Q3(60–69) | 0.55–1.79 | 6/147 | (4.1) |
| Q4(70–117) | 0.55–1.79 | 0/147 | (0.0) |
| Quartile 2 | | | |
| Q1(26–49) | 1.79–2.33 | 10/147 | (6.8) |
| Q2(50–59) | 1.79–2.33 | 13/147 | (8.8) |
| Q3(60–69) | 1.79–2.33 | 10/147 | (6.8) |
| Q4(70–117) | 1.79–2.33 | 3/147 | (2.0) |
| Quartile 3 | | | |
| Q1(26–49) | 2.36–3.08 | 8/147 | (5.4) |
| Q2(50–59) | 2.36–3.08 | 11/147 | (7.5) |
| Q3(60–69) | 2.36–3.08 | 10/147 | (6.8) |
| Q4(70–117) | 2.36–3.08 | 8/147 | (5.4) |
| Quartile 4 | | | |
| Q1(26–49) | 3.09–5.56 | 0/147 | (0.0) |
| Q2(50–59) | 3.09–5.56 | 5/147 | (3.4) |
| Q3(60–69) | 3.09–5.56 | 8/147 | (5.4) |
| Q4(70–117) | 3.09–5.56 | 24/147 | (16.3) |

PERFORMANCE CHARACTERISTICS

Detection Limit

The detection limit is <1 mU/L calculated as three standard deviations above the Calibrator 0.

Recovery

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

Precision

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

| Sample | Obtained value | | Coefficient of variation % | |
|--------|----------------|--------|----------------------------|-------|
| | mU/L | within | between | total |
| 1 | 8.5 | 5.5 | 6.2 | 8.3 |
| 2 | 19 | 7.3 | 4.0 | 8.3 |
| 3 | 32 | 6.2 | 4.0 | 7.4 |

Dilutions

| Sample | Dilution | Obtained value | Obtained/ Expected |
|----------|----------|--------------------|-----------------------|
| | | mU/L | |
| | | (Assay 1/ Assay 2) | |
| Sample 1 | 1:3321 | | |
| | 1:6642 | 19.9/18.3 | |
| | 1:13284 | 9.4/9.5 | 0.94/1.04 |
| Sample 2 | 1:3321 | – | |
| | 1:6642 | 20.6/20.4 | |
| | 1:13284 | 10.6/9.8 | 1.02/0.97 |
| Sample 3 | 1:3321 | 29.1/32.0 | |
| | 1:6642 | 15.6/15.5 | 1.07/0.97 |
| | 1:13284 | 7.7/8.0 | 1.05/1.00 |
| Sample 4 | 1:3321 | 21.6/20.2 | |
| | 1:6642 | 10.4/10.4 | 0.97/1.03 |
| | 1:13284 | 5.9/5.7 | 1.08/1.12 |
| Sample 5 | 1:3321 | 15.9/15.7 | |
| | 1:6642 | 8.1/8.0 | 1.02/1.02 |
| | 1:13284 | 4.0/4.4 | 1.02/1.13 |

Mean Obtained/Expected value is 1.03, range 0.94–1.13.

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.

REFERENCES

1. Steinberg D (1997) Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 272:20963–20966
2. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ (1995) Atherosclerosis: basic mechanisms: oxidation, inflammation, and genetics. *Circulation* 91:2488–2496
3. Steinberg D (1997) Oxidative modification of LDL and atherogenesis. *Circulation* 95:1062–1071
4. Heinecke JW (1998) Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. *Atherosclerosis* 141:1–15
5. Witztum JL, Horkko S (1997) The role of oxidized LDL in atherogenesis: immunological re-sponse and anti-phospholipid antibodies. *Ann N Y Acad Sci* 811:88–99
6. Yla-Herttuala S (1998) Is oxidized low-density lipoprotein present in vivo? *Curr Opin Lipidol* 9:337–344
7. Brown, MS, Goldstein JL (1983) Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem* 52:223–226
8. Chisolm, GM, Hazen, SL, Fox, PL, Cathcart, MK (1999) The oxidation of lipoproteins by monocyte-macrophages. *J Biol Chem* 274:25959–25962
9. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D (1998) Oxidized LDL and mal-onaldehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 98:1487–1494
10. Holvoet P, Ann Mertens, Peter Verhamme, Kris Bogaerts, Guy Beyens, Raymond Verhaeghe, Désiré Collen, Erik Muls, Frans de Werf (2001) Circulating Oxidized LDL Is a Useful Marker for Identifying Patients With Coronary Artery Disease. *Arterioscler Thromb Vasc Biol* 21:844–848
11. Holvoet P, Satssen J-M., Van Cleemput J, Collen D, Vanhaecke J (1998) Oxidized low density lipoproteins in patients with transplant-associated coronary artery disease. *Arterioscler Thromb Vasc Biol* 18:100–107
12. Holvoet P, Donck J, Landeloos M, Brouwers E, Luijckens K, Arnout J, Lesaffre E, Vanrenterghem Y, Collen D (1996) Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure. *Thromb Haemost* 76:663–669
13. Holvoet P, Van Cleemput J, Collen D, Vanhaecke J (2000) Oxidized low density lipoprotein is a prognostic marker of transplant-associated coronary artery disease. *Arterioscler Thromb Vasc Biol* 20:698–702
14. Hulthe J, Fagerberg B (2002) Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). *Arterioscler Thromb Vasc Biol* 22: 1162-1167
15. Sigurdardottir V, Fagerberg B, Hulthe J (2002) Circulating oxidized low-density lipoprotein (LDL) is associated with risk factors of the metabolic syndrome and LDL size in clinically healthy 58-year old men (AIR study). *Journal of Internal Medicine* 252:440–447

16. Kopprash S, Pietzsch J, Kuhlisch E, Fuecker K, Temelkova-Kurktschiev T, Hanefeld M, Kühne H, Julius U, Graessler J (2002) In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. *Diabetes* 51:3102–3106
17. Duntas LH, Mantzou E, Koutras DA (2002) Circulating levels of oxidized low-density lipoprotein in overt mild hypothyroidism. *Thyroid* 12:1003–1007
18. Johnston N, Lagerqvist B, Siegbahn A, Wallentin L (2002) Oxidized LDL and unstable coronary artery disease. Presented at the American Heart Association Scientific Sessions 2002 in Chicago.



SUMMARY OF PROTOCOL SHEET

| | |
|----------------------------------|---|
| Add Calibrators and samples | 25 μ L |
| Add Assay Buffer | 100 μ L |
| Incubate | 2 hour at 18–25°C on a shaker |
| Wash | 6 times |
| Add enzyme conjugate 1X solution | 100 μ L |
| Incubate | 1 hour at 18–25°C on a shaker |
| Wash | 6 times |
| Add Substrate TMB | 200 μ L |
| Incubate | 15 minutes at 18–25°C |
| Add Stop Solution | 50 μ L Shake for 5 sec to ensure mixing |
| Measure A ₄₅₀ | Evaluate results |