Mercodia Oxidized LDL ELISA

Directions for Use

10-1143-01
REAGENTS FOR 96 DETERMINATIONS

For *in vitro* diagnostic use

Gebrauchsanweisung auf Deutsch finden Sie unter folgendem Link:
Veuillez trouver le mode d'emploi en français à:
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oder/ou/o/eller/of **Fax No +46 18-570080**

Manufactured by

Mercodia AB, Sylveniusgatan 8A
SE-754 50 Uppsala,
Sweden

EXPLANATION OF SYMBOLS USED ON LABELS

Symbol	Meaning
Σ = 96	Reagents for 96 determinations
	Expire date
	Store between 2–8°C
LOT	Lot No.
IVD	For *in vitro* diagnostic use

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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. 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 *et al.* [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 15).

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.
- All samples should be handled as capable of transmitting infections.
- Each well can only be used once.
- The Stop Solution contains <5% Sulphuric acid.
  The Stop Solution is labeled:

Danger
H318 – Causes serious eye damage.
H315 – Causes skin irritation.
P280 – Wear protective gloves. Wear eye or face protection.
P264 – Wash hands thoroughly after handling.
P302 + P352 + P362 + P364 – IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.
P332 + P313 - If skin irritation occurs: Get medical attention.
P305 + P351 + P338 + P310 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

- The Enzyme Conjugate Buffer, Cal 0, 1, 2, 3, 4, 5, Wash Buffer, Assay Buffer, Sample Buffer, Control (L) and Control (H) contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).
  The Enzyme Conjugate Buffer, the Calibrators, the Wash Buffer, the Assay buffer, the Sample Buffer, Control (L) and Control (H) are labeled:

Warning
H317 – May cause an allergic skin reaction.
P280 – Wear protective gloves.
P261 – Avoid breathing vapour.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

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 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, 3.5 mL
- 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 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.

Reagent	Quantity	Volume	Preparation
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 2 months.	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 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 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 µL	7 mL
4 strips	350 µL	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. 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. 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. 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.

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 25 µL of each sample to individual tubes.
3. Add 2000 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 25 µL of each 1/81 dilution to new individual tubes.
6. Add 2000 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: 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 Coated Plate wells to accommodate Calibrators, Controls and samples in duplicate.
4. Pipette 25 µL of each Calibrator, Control and diluted sample into appropriate wells. All samples should be added to the plate within 20 minutes.
5. Add 100 µL Assay Buffer to each well.
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. 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 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. <u>Avoid prolonged soaking during washing procedure.</u>
8. Add 100 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 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, preparation dates of kit components OD values for the blank, Calibrators and Controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

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 O, versus the concentration using cubic spline regression. Multiply the concentration of the samples with the dilution factor (e.g. × 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. × 6561).

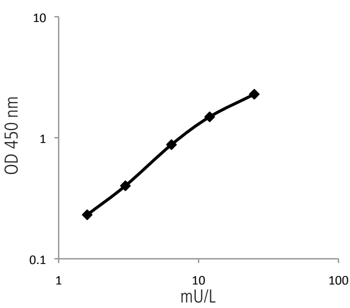
Example of results

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 (× 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

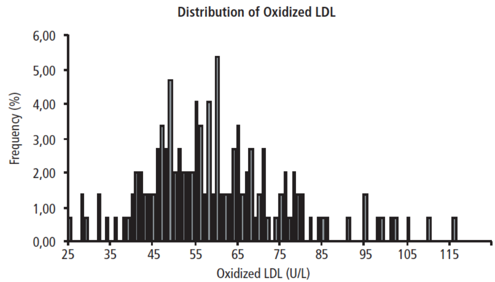
As with all diagnostic tests, a definitive diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.

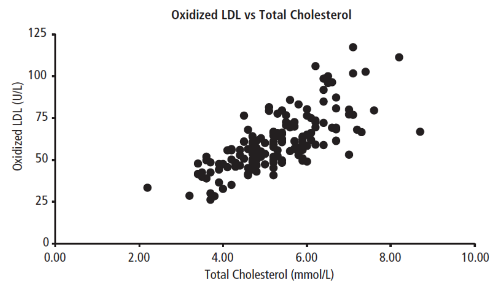
Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.

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.





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

	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/lL	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

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 0.6 mU/L as determined by the methodology described in ISO11843- Part 4.

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

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

Precision

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

		Coefficient of variation	
Sample	Obtained value mU/L	Repeatability %*	Within laboratory %**
1	8.5	5.5	8.3
2	19	7.3	8.3
3	32	6.2	7.4

*Within assay variation
**Total assay variation

Dilutions

Sample	Dilution	Obtained value mU/L	Obtained/ Expected
		(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

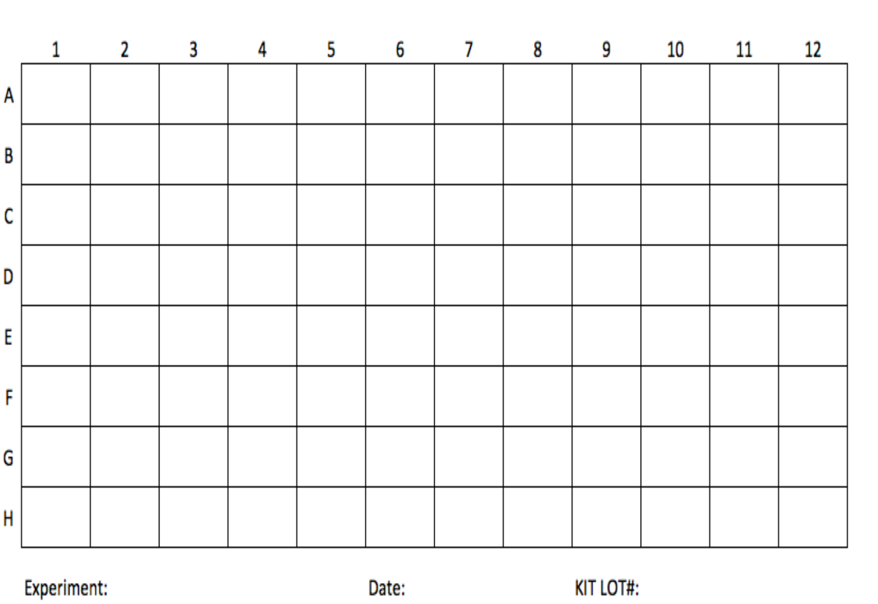
1. Steinberg D (1997) Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 272:20963–20966.
2. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD and Lusis AJ (1995) Atherosclerosis: basic mechanisms: oxidation, inflammation, and genetics. *Circulation* 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 and 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 and 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 and 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 and Collen D (1998) Oxidized LDL and mal-ondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 98:1487–1494.
10. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, Désiré Collen, Muls E and de Werf F (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 and Vanhaecke J (1998) Oxidized low den-sity 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, Luijtens K, Arnout J, Lesaffre E, Van-renterghem Y and 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 and 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 and 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 and 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). *J internal Medicine* 252:440–447.
16. Kopprash S, Pietzsch J, Kuhlisch E, Fuecker K, Temelkova-Kurktschiev T, Hanefeld M, Kühne H, Julius U and 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 and Koutras DA (2002) Circulating levels of oxidized low-density lipoprotein in overt mild hypothyrodism. *Thyroid* 12:1003–1007.
18. Johnston N, Lagerqvist B, Siegbahn A and Wallentin L (2002) Oxidized LDL and unstable coronary artery disease. Presented at the American Heart Association Scientific Sessions 2002 in Chicago, USA.

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

NOTES

NOTES



	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Experiment: Date: KIT LOT#:

SUMMARY OF PROTOCOL SHEET
Mercodia Oxidized LDL ELISA

X-O Graf Tryckeri AB

Add Calibrators, Controls and diluted samples within 20 minutes	25 µL
Add Assay Buffer	100 µL
Incubate	2 hour at 18–25°C on a shaker 700-900 rpm
Wash plate with wash buffer 1X solution	6 times
Add enzyme conjugate 1X solution	100 µL
Incubate	1 hour at 18–25°C on a shaker 700-900 rpm
Wash plate with wash buffer 1X solution	6 times
Add Substrate TMB	200 µL
Incubate	15 minutes at 18–25°C
Add Stop Solution	50 µL Shake for 5 sec to ensure mixing
Measure A_{450}	Evaluate results

For full details see page 9

For technical support please contact: support@mercodia.com

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