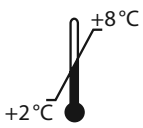
Manual

ox-LDL/MDA Adduct ELISA

For the in vitro determination of ox-LDL/MDA adducts in EDTA plasma and serum

Valid from 2017-01-12

REF **K 7810**



IVD CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 849430
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1. INTENDED USE

This Immundiagnostik assay is an enzyme immunoassay intended for the quantitative determination of ox-LDL in EDTA-plasma and serum. The test recognizes MDA-modified apolipoprotein B 100, even if it contains less than 60 MDA units per molecule.

For *in vitro* diagnostic use only.

2. INTRODUCTION

Lipid peroxidation is a natural process essential for cell growth. However, when the oxidative stress overwhelms the antioxidative cell defense, the balance is disturbed and enhanced formation of lipid peroxidation products occurs. At present, lipid peroxidation is considered to be one of the basic mechanisms involved in the initiation and progression of many diseases. Various studies have provided evidence that oxidative stress resulting in lipid peroxidation and protein modification is involved in the pathogenesis of atherosclerosis and coronary heart disease.

Lipid peroxidation products are formed during normal cell metabolism via producing an excess of free radicals that can react with unsaturated fatty acids, in particular low-density lipoprotein (LDL), the major carrier of plasma cholesterol. LDL is eliminated by macrophages. Normally, receptor-mediated uptake of LDL is suppressed through down-regulation of LDL receptor expression in response to increasing cholesterol levels. Once LDL is oxidised, it is still internalised by macrophages but through scavenger receptors whose expression is not controlled by cholesterol loading. The binding of oxidised LDL (ox-LDL) is the step by which cholesterol accumulation in macrophages is induced transforming them into lipid-loaded foam cells. This process is accompanied by extensive cell proliferation and elaboration of extra cellular matrix components and contributes to the genesis and progression of atherosclerosis by promoting endothelial damage and amplifying the inflammatory response within the vessel wall. Cholesterol-loaded macrophage foam cells are present in the earliest detectable atherosclerotic lesions, the precursor of more complex atherosclerosis that cause stenosis and limited blood flow. These advanced lesions ultimately represent the sites of thrombosis leading to myocardial infarction.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 7810	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 7810	WASHBUF	ELISA wash buffer concentrate, 10 x	2 x 100 ml
K 7810	CONJ	Conjugate concentrate, goat-anti ox-LDL, peroxidase-labelled	1 x 150 µl
K 7810	CONJBUF	Conjugate dilution buffer	1 x 15 ml
K 7810	STD	Standards, lyophilised (see specification for concentrations)	4 x 5 vials
K 7810	CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial
K 7810	CTRL2	Control, lyophilised (see specification for range)	4 x 1 vial
K 7810	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 30 ml
K 7810	SUB	TMB substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 7810	STOP	ELISA stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Laboratory balance
- Calibrated precision pipettors and 10–1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the concentrate. The crystals must be redissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solution. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised standards** (STD) and **controls** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STDs and CTRLs have to be reconstituted with **500 µl of ultra pure water**. Allow the vial content to dissolve for 10 minutes and mix thoroughly to ensure complete reconstitution. **Standards and controls** (reconstituted STDs and CTRLs) **are not stable and cannot be stored.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in conjugate dilution buffer (100 µl CONJ + 10 ml CONJBUF). The CONJ is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Venous fasting blood is suited for this test system. Samples should be stored at -20 °C up to the measurement. The maximum storage time at -20 °C is two years.

Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.

Samples with visible amounts of precipitates should be centrifuged (5 min at 3 000 *g*) prior to measurement and the resulting supernatant used in the test.

Sample preparation

EDTA plasma or serum samples must be diluted **1:10** before performing the assay, e.g. **30 µl** sample + **270 µl** SAMPLEBUF (sample dilution buffer), mix well.

For testing in duplicates, pipet **2 x 100 µl** of each prepared sample per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of ox-LDL/MDA adducts.

This assay is a sandwich ELISA for the direct measurement of ox-LDL in human EDTA plasma and serum.

Standards, controls and samples containing human ox-LDL are added to wells of microplate coated with high affinity antibodies. During the first incubation period, the antibodies immobilised on the wall of the microtiter wells capture the antigen in the patient samples. After washing away the unbound components from samples, a peroxidase-conjugated antibody is added to each microtiter well. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The intensity of the yellow colour is directly proportional to the ox-LDL concentration of sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Ox-LDL, present in the patient samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips in the aluminium packaging at 2–8 ° C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Wash the pre-coated microtiter plate (PLATE) **5 x** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
2.	Add each **100 µl standards/controls/samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a horizontal mixer.
4.	Discard the content of each well and wash **5 x** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
5.	Add **100 µl conjugate** (diluted CONJ) in each well.
6.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a horizontal mixer.
7.	Discard the content of each well and wash **5 x** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8.	Add **100 µl** TMB substrate (SUB) in each well.
9.	Incubate for **10–20 minutes*** at room temperature (15–30 °C) in the dark.
10.	Add **100 µl** ELISA stop solution (STOP) and mix well.
11.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the highest extinction of the standards (STD) is above the range of the photometer, absorption must be measured immediately at405 nm and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used.

* The intensity of the color change is temperature sensitive. We recommend observing the color change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the "4 parameter algorithm".

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

EDTA-plasma and serum

The obtained results have to be multplied with the **dilution factor of 10** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) must be further diluted and re-assayed. Please consider this greater dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

Analytical sensitivity × *sample dilution factor to be used*

10. QUALITY CONTROL

Immundiagnostik recommends the use of external controls for internal quality control, if possible.

Control samples or serum pools should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

Based on Immundiagnostik studies of evidently healthy persons (n = 77; Germany) a mean value of 287 ng/ml was estimated.

Serum/Plasma (n = 77; Germany): 287 (18–2 261) ng/ml

We recommend each laboratory to establish its own reference range. The values mentioned above are only for orientation and can deviate from other published data.

Additional reference ranges

Within a scientific study a mean value of 95.32 ± 37.85 ng ox-LDL/ml was estimated for control subjects (healthy, n=120; Tunisia) using the Immundiagnostik's ELISA Kit.

Serum/Plasma (controls, n = 120; Tunisia) 95.32 ± 37.85 ng/ml*

Furthermore, the obtained results demonstrate that a significantly elevated ox-LDL concentration (142.37 ± 49.84 ng ox-LDL/ml) was found in type 2 diabetes patients (n=86) compared with healthy controls.

In addition, higher ox-LDL values were detected in type 2 diabetes patients with hypertension, as compared with diabetic patients without hypertension.

The results of the study are summarised in the following table.

Sample	ox-LDL [ng/ml]
Controls, healthy (n = 120)	95.32 ± 37.85
Type 2 diabetes patients (n = 86)	142.37 ± 49.84
Type2 diabetes patients without hypertension	111.16 ± 33.42
Type 2 diabetes patients with hypertension	157.4 ± 49.9

*Koubaa N et al. (2007) Clin. Biochem. 40, 1007-1014

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Two highly positive patient samples were diluted 1:120 or 1:160 and measured using the assay.

Intra-Assay (n = 18)

Sample	ox-LDL [ng/ml]	CV [%]
1	3678.024	3.9
2	6452.786	5.7

Inter-Assay (n = 14)

Sample	ox-LDL [ng/ml]	CV [%]
1	7202.643	11.0
2	4108.071	9.0

Spiking Recovery

Two samples were spiked with 3 different ox-LDL standards concentrations and measured using this assay (n = 2).

Sample	Unspiked Sample [ng/ml]	Spike [ng/ml]	ox-LDL expected [ng/ml]	ox-LDL measured [ng/ml]
A	31.4	10.0	41.4	39.6
	31.4	25.0	56.4	56.0
	31.4	30.0	61.4	59.3
B	22.5	12.5	35.0	31.1
	22.5	25.0	47.5	43.6
	22.5	50.0	72.5	73.8

Dilution recovery

Two patient samples were diluted and analysed. The results are shown below (n = 2):

Sample	Dilution	ox-LDL expected [ng/ml]	ox-LDL measured [ng/ml]
A	1:15	3503.00	3503.00
	1:30	1751.50	1827.50
	1:60	875.75	920.25
	1:120	437.875	477.50
B	1:40	7867.00	7867.00
	1:80	3933.50	3868.00
	1:160	1966.75	2000.75
	1:320	983.375	952.625

Analytical Sensitivity

The Zero-standard was measured 22 times. The detection limit was set as B_0 + 2 SD and estimated to be 4.130 ng/ml.

Sample	ox-LDL mean value [OD]	Standard variation (2 SD)	Detection limit [ng/ml]
1	0.140	0.024	4.130

11. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or Thimerosal as bactericides. Sodium azide and Thimerosal are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any

spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

12. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according the enclosed manual.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

14. REFERENCES

1. Viereck, Volker, Carsten Gründker, Sabine Blaschke, Britta Niederkleine, Heide Corsi MM, Dogliotti G, Pedroni F, Ermetici F, Malavazos A, Ambrosi B, (2007) ADMA: a possible role in obese patients. *Poster P173 of the 6th World Congress on Hyperhomocysteinemia*, Saarbrücken, Germany, June 5-9, 2007, erschienen in CCLM **45**(5)
2. Koubaa N, Nakbi A, Smaoui M, Abid N, Chaaba R, Abid M, Hammami M (2007) Hyperhomocysteinemia and elevated ox-LDL in Tunisian type 2 diabetic patients: Role of genetic and dietary factors. *Clin Biochem* **40**(13-14):1007-14. Epub 2007 Jun 14
3. Licastro F, Dogliotti G, Goi G, Malavazos AE, Chiappelli M, Corsi MM (2007) Oxidated low-density lipoproteins (oxLDL) and peroxides in plasma of Down syndrome patients. *Arch Gerontol Geriatr* **44** Suppl 1:225-32
4. Pfützner A, Kost I, Löbig M, Knesovic M, Armbruster FP, Forst T (2005) Clinical Evaluation of a New ELISA Method for Determination of Oxidized LDL Particles - a Potential Marker for Arteriosclerotic Risk in Diabetes Mellitus. *Abstract of the 5th Diabetes Technology Meeting*, San Francisco, 10.-12. November 2005

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention

Immundiagnostik AG

Stubenwald-Allee 8a
D-64625 Bensheim

Tel.: +49 (0) 62 51/70 19 00
Fax: +49 (0) 62 51/84 94 30

info@immundiagnostik.com
www.immundiagnostik.com