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Manual

Anti ox-LDL ELISA

For the in vitro determination of anti ox-LDL antibodies in EDTA plasma and serum

Valid from 2021-07-27

REF **K 7809**

Σ 96

+2°C +8°C

IVD

CE

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of ox-LDL antibodies in EDTA-plasma and serum. 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 particularly 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.

Oxidised LDL is not only an essential trigger of arteriosclerosis and vascular ageing. When modified by the oxidation LDL becomes immunogenic. Specific auto antibodies against epitopes (e.g. malondialdehyde-Lysine) of oxidised LDL in serum have been detected. Auto antibodies against oxidised low density lipoprotein have been found in sera of patients with a number of different symptoms, like diabetes, vascular diseases, carotid arteriosclerosis and others. The ox-LDL antibodies recognise tissues with atherosclerotic lesions. The titer of oxidised LDL antibodies is considered as an independent indicator for the progress of atherosclerosis.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 7809	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 7809	STD	anti ox-LDL standard concentrate, lyophilised	4 x 1 vial
K 7809	CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial
K 7809	CTRL2	Control, lyophilised (see specification for range)	4 x 1 vial
K 7809	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 200 µl
K 7809	SAMPLEBUF	Sample dilution buffer, ready-to-use	2 x 100 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	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

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

* Immundiagnostik AG recommends the use of ultrapure 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. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. 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 **for 1 month at 2–8 °C**.
- The **lyophilised standard concentrate** (STD) and **controls** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details and the preparation of the standard curve are given in the **specification data sheet**. **Standard concentrate and controls** (reconstituted STD and CTRL) **are not stable and cannot be stored.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until 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) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

EDTA plasma and serum

Venous fasting blood is suited for this test system. Samples should be stored at -20 °C up to the measurement.

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.

The EDTA plasma and serum samples must be diluted **1:10 000** in **sample dilution buffer** (SAMPLEBUF) prior to analyses, e. g.

10 µl sample + **990 µl** SAMPLEBUF, mix well = dilution I (1:100)

10 µl dilution I + **990 µl** SAMPLEBUF, mix well = dilution II (1:10 000)

For testing in duplicates, pipette **2 x 100 µl of dilution II per well.**

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of ox-LDL antibodies.

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

Standards, controls and samples containing human anti ox-LDL antibodies are added into the wells of a microplate coated with malondialdehyde-modified low-density lipoprotein (MDA-LDL, ox-LDL). During the first incubation period, ox-LDL immobilised on the wall of the microtiter wells, captures the antibodies in the samples. After washing away the unbound components, a peroxidase-labelled conjugate is added into each microtiter well. Tetramethylbenzidine is used as a peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The intensity of the yellow colour is directly proportional to the ox-LDL antibody concentration of the sample. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. The ox-LDL antibody concentration in the 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 kit. Store unused strips together with the desiccant bag in the closed 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.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips tightly and incubate for **2 hours** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl conjugate** (diluted CONJ) into each well.
6.	Cover the strips tightly and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well 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 extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour 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

For the calculation of the concentration of ox-LDL antibodies in EDTA-plasma and serum samples, the result should be multiplied by **10 000**.

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

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher 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:

LoB × *sample dilution factor to be used*

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

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

Control samples 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 AG in-house studies of serum and plasma samples (plasma n = 76 and serum n = 69)of apparently healthy persons, a mean reference range of 4 000–12 000 U/ml was estimated.

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 40)

The precision (intra-assay variation) was calculated from 40 replicate determinations on each one of three samples.

Sample	anti ox-LDL [U/ml]	CV [%]
1	0.723	6.866
2	0.309	4.232
3	0.147	5.343

Inter-Assay (n = 10)

The total precision (inter-assay variation) was calculated from data on 2 samples obtained in 10 different assays.

Sample	anti ox-LDL [U/ml]	CV [%]
1	0.150	11.895
2	0.257	14.391

Analytical Sensitivity

The LoB (limit of blank) was evaluated according to the guideline CLSI EP17-A2 and resulted in 0.0205 U/ml.

12. 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 ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact

Warning: Causes serious eye irritation

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.

- 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.

13. 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 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 to the enclosed manual.

14. 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.

15. REFERENCES

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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


Consult instructions for use

Consult specification data sheet