Manual

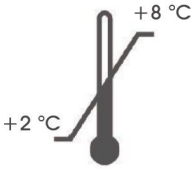
Vorläufig/Preliminary

Anti ox-LDL ELISA Kit

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

Valid from 07.01.2011

K 7809



1. INTENDED USE

The ox-LDL Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the quantitative determination of anti ox-LDL antibodies in EDTA-plasma and serum. It is for research 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 oxidized, it is still internalized by macrophages but through scavenger receptors whose expression is not controlled by cholesterol loading. The binding of oxidized 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.
Oxidized 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 oxidized LDL in serum have been detected. Auto antibodies against oxidized 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 anti ox-LDL antibodies recognize tissues with atherosclerotic lesions. The titer of oxidized LDL antibodies is considered as an independent indicator for the progress of atherosclerosis.

3. PRINCIPLE OF THE TEST

This assay is a sandwich ELISA for the direct measurement of anti 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 ox-LDL. During the first incubation period, ox-LDL immobilized on the wall of the microtiter wells, captures the antibodies in the samples. After washing away the unbound components, a peroxidase-labeled conjugate is added into each microtiter well. Tetramethylbenzidine (TMB) is used as a peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The intensity of the yellow color is directly proportional to the anti ox-LDL antibodies 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. Anti ox-LDL antibody concentration in the samples is determined directly from this curve.

4. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
K7809MTP	PLATE	One holder with precoated strips	12 x 8 wells
K7809WP	WASHBUF	Wash buffer concentrate (10 fold)	2 x 100 ml
K7809ST	STD	anti ox-LDL Standard concentrate (lyophilized)	4 vials
K7809KO	CTRL	Control (lyophilized)	4 vials
K7809K	CONJ	Conjugate, concentrate	1 x 200 µl
K7809PV	SAMPLEBUF	Sample dilution buffer, ready to use	2 x 100 ml
K7809TMB	SUB	TMB substrate, ready-to-use	1 x 15 ml
K7809AC	STOP	Stop solution, ready-to-use	1 x 15 ml

5. Material Required but not supplied

- Bidistilled water (aqua bidest.)
- Laboratory balance
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm
  (reference wave length 620 or 690 nm)

6. Preparation and storage of reagents

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. 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.

- The **ELISA WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution. The **WASHBUF** (buffer concentrate) is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.

- The lyophilized **STD** (Standard concentrate) and **CTRL** (control) are stable at 2-8°C until expiry date stated on the label. Reconstitution details are given in the data sheet.

  Use the reconstituted **anti ox-LDL STD** (Standard concentrate) to prepare dilution series for the calibration curve with **SAMPLEBUF** (sample dilution buffer) **in 1:3 dilution steps**:

200 µl STD + **400 µl** SAMPLEBUF = **S5**

200 µl S5 + **400 µl** SAMPLEBUF = **S4**

200 µl S4 + **400 µl** SAMPLEBUF = **S3**

200 µl S3 + **400 µl** SAMPLEBUF = **S2**

SAMPLEBUF is used as Standard S1 (0 ng/ml).

- The **CONJ** (conjugate) must be diluted **1:101** in **wash buffer** (100 µl CONJ + 10 ml wash buffer). The undiluted conjugate is stable at **2-8 °C** until expiry date stated on the label. **Diluted conjugate is not stable and can not 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**.

7. PRECAUTIONS

- For research 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 if potentially infectious.
- Stop solution is composed of sulfuric acid, which is a strong acids acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on kit label.

8. SPECIMEN COLLECTION AND PREPARATION

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 10000 g) prior to measurement and the resulting supernatant used in the test.

- The **EDTA-plasma and serum** samples should be diluted **1:50000** with SAMPLEBUF (sample dilution buffer) prior to analyses, e.g.:

  50 µl sample + 200 µl SAMPLEBUF = dilution I

  10 µl dilution I + 990 µl SAMPLEBUF = dilution II

  10 µl dilution II + 990 µl SAMPLEBUF = dilution III

  For analysis, pipet **100 µl** of dilution step III per well.

9. ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Immundiagnostik can therefore not be held reliable for any damage resulting from this.
- The assay should always be performed according to the enclosed manual.

Test procedure

1.	Bring all reagents and samples to **room temperature** (18-26 °C) and mix well
2.	Mark the **positions of STD /SAMPLE/CTRL** (Standards/Sample/Control) in duplicate on a protocol sheet
3.	Take as many **microtiter strips** as needed from kit. Store unused strips in the original package bag at 2-8° C. Strips are stable until the expiry date stated on the label
4.	Wash each well **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper

5.	Add **100 µl of STD/SAMPLE/CTRL** (Standard/Sample/Control) in duplicate into respective well
6.	Cover plate tightly and **incubate for 2 hours at room temperature** (18-26°C) on a horizontal mixer
7.	Aspirate the contents of each well. Wash each well **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
8.	Add **100 µl diluted conjugate** into each well
9.	Cover plate tightly and **incubate for 1 hour at room temperature** (18-26°C) on a horizontal mixer
10.	Aspirate the contents of each well. Wash each well **5 times by dispensing 250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
11.	Add **100 µl of SUB** (substrate) into each well
12.	Incubate for **10 - 20 minutes at room temperature** (18-26°C) in the dark*
13.	Add **50 µl of STOP** (stop solution) into each well, mix thoroughly
14.	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

*The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

10. EVALUATION OF RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-parameter-algorithm

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

2. Point-to-point-calculation

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

3. Spline-algorithm

   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

   The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done 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 **50 000**.

Controls

Control samples or serum pools should be analyzed with each run. Results, generated from the analysis of the 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.

11. LIMITATIONS

Strong hemolytic and lipemic samples often show wrong concentrations. **Do not** analyze such samples.

12. REFERENCES (based on the ox-LDL ELISA of Immundiagnostik)

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)

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

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

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

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- For research use only.
- Quality control guidelines should be followed.
- 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 color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- The assay should always be performed according the enclosed manual.
- 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 wrong use.
- Warranty claims and complaints in respect of 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.

Used symbols:


Temperature limitation

REF Catalogue Number

RUO For research use only


Contains sufficient for <n> tests


Manufacturer


Use by

LOT Lot number