ox-LDL/MDA Adduct ELISA Kit

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

Valid from 31.01.2012

REF K 7810

+2 °C
+8 °C

CE IVD
1. INTENDED USE

The ox-LDL Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the quantitative determination of ox-LDL in EDTA-plasma and serum. The test recognizes MDA-modified Apolipoprotein B 100, containing less than 60 MDA units per molecule. It is for in vitro diagnostic use only.

2. INTRODUCTION

Lipid peroxidation is a natural process essential for cell growth. However, when the oxidative stress overpowers 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 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 extracellular 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. **PRINCIPLE OF THE TEST**

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 immobilized 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 color is directly proportional to the ox-LDL concentration of sample. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated; using the values obtained from standard. Ox-LDL present in the patient samples is determined directly from this curve.

4. **MATERIAL SUPPLIED**

<table>
<thead>
<tr>
<th>Catalog No</th>
<th>Label</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K7810MTP</td>
<td>PLATE</td>
<td>One holder with precoated strips</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>K7810STD</td>
<td>STD</td>
<td>Standards (lyophilized)</td>
<td>4 x 5 vials</td>
</tr>
<tr>
<td>K7810KO1</td>
<td>CTRL</td>
<td>Control (lyophilized)</td>
<td>4 x 1 vial</td>
</tr>
<tr>
<td>K7810KO2</td>
<td>CTRL</td>
<td>Control (lyophilized)</td>
<td>4 x 1 vial</td>
</tr>
<tr>
<td>K7810WB</td>
<td>WASHBUF</td>
<td>Wash buffer concentrate (10 fold)</td>
<td>2 x 100 ml</td>
</tr>
<tr>
<td>K7810K</td>
<td>CONJ</td>
<td>Conjugate, (goat-anti ox-LDL, peroxidase-labeled)</td>
<td>150 μl</td>
</tr>
<tr>
<td>K7810PV</td>
<td>SAMPLEBUF</td>
<td>Sample dilution buffer</td>
<td>50 ml</td>
</tr>
<tr>
<td>K7810TMB</td>
<td>SUB</td>
<td>TMB substrate</td>
<td>15 ml</td>
</tr>
<tr>
<td>K7810AC</td>
<td>STOP</td>
<td>Stop solution</td>
<td>15 ml</td>
</tr>
</tbody>
</table>
5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- 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

*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 < 0.055 μS/cm at 25°C (≥18.2 MΩ cm).

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 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 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.
- STD (Standards) and CTRL (controls) must be reconstituted with 500 μl of ultra pure water. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted standards and controls are not stable.
- 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 *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 if potentially infectious.
• 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.
• 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. 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 10000 g) prior to measurement and the resulting supernatant used in the test.
• The **EDTA-plasma and serum** samples should be diluted **1:10** with sample dilution buffer prior to analyses.
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 liable 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/Controls) 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/Controls) in duplicate into respective well

6. Cover plate tightly and incubate for 1 hour 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 **100 μl of STOP (stop solution)** into each well, mix thoroughly.

14. Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards (STD) is above the range of the photometer, absorption must be measured immediately at **405 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 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 ox-LDL concentration in EDTA-plasma and serum samples, the result has to be multiplied by 10.

Expected values

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 – 2261) ng/ml

Within a scientific study a mean value of $95.32 \pm 37.85$ 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):** $95.32 \pm 37.85$ ng/ml*

Furthermore, the obtained results demonstrate that a significantly elevated ox-LDL concentration ($142.37 \pm 49.84$ ng ox-LDL/ml) was found in Typ-2 Diabetes patients (n=86) compared with healthy controls.
In addition, higher ox-LDL values were detected in Typ-2 Diabetes patients with hypertension, as compared with diabetic patients without hypertension.

The results of the study are summarized in the following tables.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ox-LDL [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, healthy (n=120)</td>
<td>95.32 ± 37.85</td>
</tr>
<tr>
<td>Typ-2 Diabetes patients (n=86)</td>
<td>142.37 ± 49.84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ox-LDL [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typ-2 Diabetes patients without hypertension</td>
<td>111.16 ± 33.42</td>
</tr>
<tr>
<td>Typ-2 Diabetes patients with hypertension</td>
<td>157.4 ± 49.9</td>
</tr>
</tbody>
</table>


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

**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. PERFORMANCE CHARACTERISTICS

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

<table>
<thead>
<tr>
<th>Intra-Assay (n=18)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Ox-LDL [ng/ml] CV [%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 3678.024 3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 6452.786 5.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-Assay (n=14)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Ox-LDL [ng/ml] CV [%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 7202.643 11.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 4108.071 9.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Recovery
Two samples were spiked with 3 different Ox-LDL standards and measured using this assay.

<table>
<thead>
<tr>
<th>Sample [ng/ml]</th>
<th>Spike [ng/ml]</th>
<th>Ox-LDL expected [ng/ml]</th>
<th>Ox-LDL measured [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.4</td>
<td>10.0</td>
<td>41.4</td>
<td>39.6</td>
</tr>
<tr>
<td>31.4</td>
<td>25.0</td>
<td>56.4</td>
<td>56.0</td>
</tr>
<tr>
<td>31.4</td>
<td>30.0</td>
<td>61.4</td>
<td>59.3</td>
</tr>
<tr>
<td>22.5</td>
<td>12.5</td>
<td>35.0</td>
<td>31.1</td>
</tr>
<tr>
<td>22.5</td>
<td>25.0</td>
<td>47.5</td>
<td>43.6</td>
</tr>
<tr>
<td>22.5</td>
<td>50.0</td>
<td>72.5</td>
<td>73.8</td>
</tr>
</tbody>
</table>
Sensitivity
The sensitivity was set as $B_0 + 2$ SD. The zero-standard was measured 22 times.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ox-LDL mean value [OD]</th>
<th>Standard variation (2 SD)</th>
<th>Detection limit [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.140</td>
<td>0.024</td>
<td>4.130</td>
</tr>
</tbody>
</table>

Linearity
Two patient samples were diluted with sample buffer and analyzed. The results are shown below:

$n = 2$

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Expected [ng/ml]</th>
<th>Measured [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1:15</td>
<td>3503.00</td>
<td>3503.00</td>
</tr>
<tr>
<td></td>
<td>1:30</td>
<td>1751.50</td>
<td>1827.50</td>
</tr>
<tr>
<td></td>
<td>1:60</td>
<td>875.75</td>
<td>920.25</td>
</tr>
<tr>
<td></td>
<td>1:120</td>
<td>477.50</td>
<td>437.875</td>
</tr>
<tr>
<td>B</td>
<td>1:40</td>
<td>7867.00</td>
<td>7867.00</td>
</tr>
<tr>
<td></td>
<td>1:80</td>
<td>3933.50</td>
<td>3868.00</td>
</tr>
<tr>
<td></td>
<td>1:160</td>
<td>1966.75</td>
<td>2000.75</td>
</tr>
<tr>
<td></td>
<td>1:320</td>
<td>983.375</td>
<td>952.625</td>
</tr>
</tbody>
</table>

12. LIMITATIONS

Strong haemolytic and lipaemic samples often show wrong concentrations. We recommend not to measure those samples.
13. REFERENCES (based on the ox-LDL ELISA of Immundiagnostik)


14. 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 *in vitro* diagnostic 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:

- IVD: In Vitro Diagnostic Medical Device
- Manufacturer
- LOT: Lot number
- REF: Catalogue Number
- Temperature limitation
- Contains sufficient for <n> tests
- Use by