Manual

ox-LDL ELISA

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

For laboratory professional use only

Valid from 2023-02-23

REF **K 7810.US**
K 7810.20.US

Σ 96
Σ 20 x 96

+8 °C
+2 °C

IVD

Rx Only

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1. INTENDED USE

This Immundiagnostik AG assay is a quantitative test system intended for the measurement of oxidized low density lipoprotein (ox-LDL) in EDTA-plasma and serum.

For *in vitro* diagnostic use only. For laboratory professional use only.

2. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity for cat. no.	
			K 7810.US	K 7810.20.US
K 7810	PLATE	Microtiter plate, pre-coated	12 x 8 wells	20 x 12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml	20x 100 ml
K 7810	CONJ	Conjugate concentrate, goat-anti ox-LDL, peroxidase-labelled	1 x 150 µl	20 x 150 µl
K 7810	CONJBUF	Conjugate dilution buffer, ready-to-use	1 x 15 ml	20 x 15 ml
K 7810	STD	Standards, lyophilised (see specification for concentrations)	4 x 5 vials	25 x 5 vialsl
K 7810	CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial	25 x 1 vial
K 7810	CTRL2	Control, lyophilised (see specification for range)	4 x 1 vial	25 x 1 vial
K 7810	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 30 ml	25 x 30 ml
K 0002.15	SUB	Substrate (tetra-methylbenzidine), ready-to-use	1 x 15 ml	20 x 15 ml

Cat. No.	Label	Kit components	Quantity for cat. no.	
			K 7810.US	K 7810.20.US
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml	20 x 15 ml

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

3. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1 000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3 000 *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).

4. 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 ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure 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 STD and CTRL have to be reconstituted with **500 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Standards 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 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) when stored at **2–8 °C**.

5. STORAGE AND PREPARATION OF SAMPLES

EDTA-plasma and serum

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** sample dilution buffer (SAMPLEBUF), mix well.

100 µl of the dilution are used in the test per well.

6. ASSAY PROCEDURE

Principle of the test

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

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 samples. After washing away the unbound components from samples, a peroxidase-conjugated antibody is added to each microtiter well. Tetramethyl-benzidine (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 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 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/prepared samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** 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 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.

7. 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 by the **dilution factor of 10** to get the actual concentrations.

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

8. 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:

Analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity see chapter "Performance Characteristics".

9. QUALITY CONTROL

Immundiagnostik AG 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 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

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 42

The repeatability was assessed with 2 plasma samples under **constant** parameters (same operator, instrument, day and kit lot). The results below were obtained without consideration of the sample dilution factor.

Sample	Mean value [ng/ml]	CV [%]
1	30.65	3.9
2	40.33	5.7

Reproducibility (Inter-Assay); n = 15

The reproducibility was assessed with 2 plasma samples under **varying** parameters (different operators, instruments, days and kit lots). The results below were obtained without consideration of the sample dilution factor.

Sample	Mean value [ng/ml]	CV [%]
1	45.35	11.8
2	34.07	9.9

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, ox-LDL spikes with known concentrations were added to 2 different plasma samples. The results below were obtained without consideration of the sample dilution factor.

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
31.4	10.0	41.4	39.6	95.65
	25.0	56.4	56.0	99.29
	30.0	61.4	59.3	96.58

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
22.5	12.5	35.0	31.1	88.86
	25.0	47.5	43.6	91.79
	50.0	72.5	73.8	101.79

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP06-A with a serial dilution of 2 different plasma and 1 serum sample.

For ox-LDL in serum and plasma, the method has been demonstrated to be linear from 6.27 to 273.00 ng/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
Plasma 1	1:15	200.50	200.50	100.00
	1:30	100.25	96.87	96.63
	1:60	50.12	49.95	99.65
	1:120	25.06	26.79	106.88
	1:240	12.53	14.55	116.09
	1:480	6.27	5.94	94.81
Plasma 2	1:100	273.00	273.00	100.00
	1:200	136.50	143.50	105.13
	1:400	68.25	71.25	104.40
	1:800	34.13	32.25	94.51
	1:1 600	17.06	16.88	98.90
	1:3 200	8.53	9.91	116.12

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
Serum	1:20	190.59	190.59	100.00
	1:40	91.37	95.30	95.88
	1:80	39.82	47.65	83.57
	1:160	18.86	23.82	79.16
	1:320	9.10	11.91	76.39

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB 5.836 ng/ml

Limit of detection, LoD 6.645 ng/ml

Limit of quantitation, LoQ 6.645 ng/ml

The evaluation was performed according to the CLSI guideline EP17-A2. The specified accuracy goal for the LoQ was 20 % CV.

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to ox-LDL. There was no cross-reactivity observed.

Substance tested	Concentration added	Concentration obtained [ng/ml]	Conclusion
HDL (High-Density-Lipoproteine)	10 500 ng/ml	< 5.836	< LoB
LDL (Low-Density-Lipoproteine)	14 400 ng/ml	< 5.836	< LoB
Albumin	800 ng/ml	< 5.836	< LoB
AOPP (Advanced oxidation protein products)	100 µmol/l	< 5.836	< LoB

11. PRECAUTIONS

- 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 or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- 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 should 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 to the enclosed manual.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- 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.

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


Irritant

Prescription use only

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