Human Oxidized LDL (oxLDL) ELISA Kit

PromoKine

Instruction Manual

Cat. No. PK-EL-K7810

Contents

Intended Use

This PromoCell ELISA is an enzyme immunoassay intended for the quantitative determination of ox-LDL in EDTA-plasma, serum and dried blood spots. The test recognises MDA-modified apolipoprotein B 100, even if it contains less than 60 MDA units per molecule.
For *in vitro* research use only

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.

Material Supplied

Label	Kit Components	Quantity
PLATE	Microtiter plate, pre-coated	12 x 8 wells
WASHBUF	Wash buffer concentrate,10 x	2 x 100 ml
CONJ	Conjugate concentrate, goat-anti ox-LDL, peroxidase-labeled	150 µl
CONJBUF	Conjugate dilution buffer, ready-to-use	15 ml
STD	Standards, lyophilized	4 x 5 vials
CTRL1	Control, lyophilized	4 x 1 vial
CTRL2	Control, lyophilized	4 x 1 vial
SAMPLEBUF	Sample dilution buffer	30 ml
SUB	Substrate (tetramethylbenzidine), ready-to-use	15 ml
STOP	Stop solution, ready-to-use	15 ml

Material Required But Not Supplied

- Ultra pure water*
- Dried blood spot carrier
- 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, 3000g
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* PromoCell 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 >0.2µm) with an electrical conductivity of 0.055µS/cm at 25°C (≥18.2MΩcm).

Preparation and Storage 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 **100µl** 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 (100ml WASHBUF + 900ml 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 STD and CTRL have to be reconstituted with **500µl of ultra pure 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 (100µl CONJ + 10ml 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.**

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.

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 10,000 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. **30µl** sample + **270µl** sample dilution buffer (SAMPLEBUF), mix well.

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

Dried blood spots

Collection and storage of dried blood spots

50µl whole blood dripped on a dried sample carrier are suitable as sample material after complete drying. We recommend DrySpotID as dried blood spot carrier. The moistened cards are stable for 3 weeks at room temperature.

Preparation of the dried blood samples

- Label 1,5-ml polypropylene tubes.
- Remove filter from sampling device.
- Put filter in a labelled tube.
- Add **300 µl** sample dilution buffer (SAMPLEBUF) to each sample, allow sample to stand for **20 min** at room temperature (15-30°C).
- Vortex for **10s.** The filter will decolourise.
- Centrifuge the sample for **5 min** at **3000 *g*** to remove residual filter pieces.

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

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 450nm) 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 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 PromoCell.

We recommend carrying 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 irmly tapping the plate on absorbent paper.
8. Add **100 µl substrate** (SUB) into each well.
9. Incubate for **10–20min**** 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.

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

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 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 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, serum and dried blood spots

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.

Limitations

Samples with concentrations above the measurement range (see definition below) can 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*

Analytical sensitivity see chapter "Performance Characteristics"

Quality Control

PromoCell 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 studies of evidently heathy persons (n=77; Germany) a mean value of 287 ng/ml was estimated

Serum/Plasma (n = 77; Germany): 287 (41–2261) ng/ml

Median 141 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 ± 37.85ng ox-LDL/ml was estimated for control subjects (healthy, n=120; Tunisia) using the PromoCell's ELISA Kit.

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

Furthermore, the obtained results demonstrate that a significantly elevated ox-LDL concentration (142.37 ± 49.84ng 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
Type 2 diabetes patients without hypertension	111.16 ± 33.42
Type 2 diabetes patients with hypertension	157.4 ± 49.9

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

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	353.00	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

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

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

Analytical Sensitivity

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

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

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 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 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.

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

General Notes on the Test and Test Procedure

- All reagents in the kit package are for *in vitro* research use only.
- The quality control guidelines should be observed.
- 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. PromoCell 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 sent to PromoCell GmbH along with a written complaint.

Ordering Information

Product Name	Product Description	Size	Catalog Number
Oxidized LDL (oxLDL) ELISA Kit, human	Human Oxidized LDL (oxLDL) ELISA Kit	96 Tests	PK-EL-K7810

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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