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ab242302 Oxidized LDL Assay Kit (MDA-LDL, Human) ELISA Kit

View Oxidized LDL Assay Kit (MDA-LDL, Human) ELISA Kit datasheet:
www.abcam.com/ab242302

For the quantitative measurement of MDA-LDL in plasma, serum or other biological fluid samples.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Oxidized LDL Assay Kit (MDA-LDL, Human) ELISA Kit (ab242302) is developed for the detection and quantitation of oxidized phospholipids (OxPL) associated with human LDL in plasma, serum or other biological fluid samples.

The kit contains an OxLDL Standard and has a detection sensitivity limit of <15 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

2. Protocol Summary

Prepare all reagents, samples and standards as instructed.

Add 100 µL of OxLDL standard or sample to the anti-MDA Antibody coated plate. Cover and incubate for 2 hrs at room temperature.

Follow washing steps and add 100 µL of diluted Blocking reagent to each well, incubate for 1 hour.

Follow washing steps with 1X Wash Buffer. Then add 100 µL diluted Biotinylated Anti-Human ApoB-100 antibody to each well. Incubate for 1 hr at room temperature.

Washing steps with 1X Wash Buffer. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate for 1 hr at room temperature.

Washing steps with 1X Wash Buffer. Add 100 µL warm Substrate Solution to each well. Incubate for 5-20 mins at room temperature.

Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Read absorbance immediately on a microplate reader using 450 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Anti-MDA Antibody Coated Plate	96 well	+4°C
Biotinylated Anti-Human ApoB-100 Antibody (1000X)	20 µL	+4°C
LDL Precipitation Solution (2X)	20 mL	+4°C
Streptavidin-Enzyme Conjugate	20 µL	+4°C
Assay Diluent	50 mL	+4°C
10X Wash Buffer	100 mL	+4°C
Substrate Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
OxLDL Standard	25 µL	+4°C
Blocking Reagent (100X)	200 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at O.D. 450 nm (620 nm as optional reference wave length)

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 1X Wash Buffer:

6.1.1 Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.

6.2 Biotinylated Anti-Human ApoB-100 Antibody:

6.2.1 Immediately before use dilute the Anti-ApoB-100 antibody 1:1000 with Assay Diluent.

6.3 Streptavidin-Enzyme Conjugate:

6.3.1 Immediately before use dilute the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent.

6.4 Blocking Reagent:

6.4.1 Immediately before use dilute the Blocking Reagent 1:100 with PBS. Do not store diluted solutions.

Δ Note: Do not store diluted solutions.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Prepare a dilution series of OxLDL Standards in the concentration range of 0 to 1 µg/mL in Assay Diluent as shown in the table below:

Standard #	0.5 mg/mL OxLDL-Standard (µL)	Assay Diluent (µL)	Final OxLDL Standard (ng/mL)
1	2	998	1000
2	250 of standard #1	250	500
3	250 of standard #2	250	250
4	250 of standard #3	250	125
5	250 of standard #4	250	62.5
6	250 of standard #5	250	31.25
7	250 of standard #6	250	15.63
8	0	250	0

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

8.1 **Plasma:** Collect blood with heparin or EDTA and centrifuge for 10 mins at 1000 *x g* at 4°C. Remove 200 µL of plasma and add 200 µL of LDL Precipitation Solution, mixing well. Incubate at room temperature for 5 mins (precipitation will occur). Centrifuge for 20 mins at 2000 *x g* (pellet should be visible). Carefully aspirate the supernatant and collect the pellet. Resuspend and dissolve the pellet in 1.6 mL of PBS, vortexing well.

8.2 **Serum:** Harvest serum and centrifuge for 10 mins at 1000 *x g* at 4°C. Remove 200 µL of serum and add 200 µL of LDL Precipitation Solution, mixing well. Incubate at room temperature for 5 mins (precipitation will occur). Centrifuge for 20 mins at 2000 x g (pellet should be visible). Carefully aspirate the supernatant and collect the pellet. Resuspend and dissolve the pellet in 1.6 mL of PBS, vortexing well.

Δ Note: Assay immediately and do not store solutions.

Δ Note: Further dilute the samples 1:200 to 1:1000 in Assay Diluent before running the ELISA.

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

9.1 For plasma and serum samples, refer to the above Sample Preparation section. These samples require LDL Precipitation Solution treatment immediately prior to running the assay.

9.2 Add 100 µL of OxLDL standard or unknown sample to the Anti-MDA Antibody Coated Plate.

9.3 Cover with a plate cover and incubate at room temperature for 2 hrs on an orbital shaker.

9.4 Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

9.5 Add 100 µL of diluted Blocking Reagent to each well. Cover with a plate cover and incubate at RT for 1 hr on an orbital shaker.

9.6 Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

9.7 Add 100 µL of the diluted Biotinylated Anti-Human ApoB-100 antibody to each well. Incubate at room temperature for 1 hr on an orbital shaker.

9.8 Wash the strip wells 5 times according to step 9.6 above.

9.9 Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hr on an orbital shaker.

9.10 Wash the strip wells 5 times according to step 9.6 above.

9.11 Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 mins.

Δ Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9.12 Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).

9.13 Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

10. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

10.1 Average the duplicate reading for each standard, control and sample.

10.2 Subtract the mean value of the blank (Standard #8) from all standards, controls and sample readings. This is the corrected absorbance.

10.3 If significant, subtract the sample background control from sample readings.

10.4 Plot the corrected values for each standard as a function of the final concentration of MDA-LDL.

10.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

10.6 Apply the corrected sample OD reading to the standard curve to get MDA-LDL amount in the sample wells.

10.7 Concentration of MDA-LDL in [B units / V units] in the test samples is calculated as:

$$MDA\ LDL\ concentration = \frac{B}{V} * D$$

Where:

B = amount of MDA-LDL in the sample well calculated from standard curve in µL/mL

V = sample volume added in the sample wells in µL

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

11. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

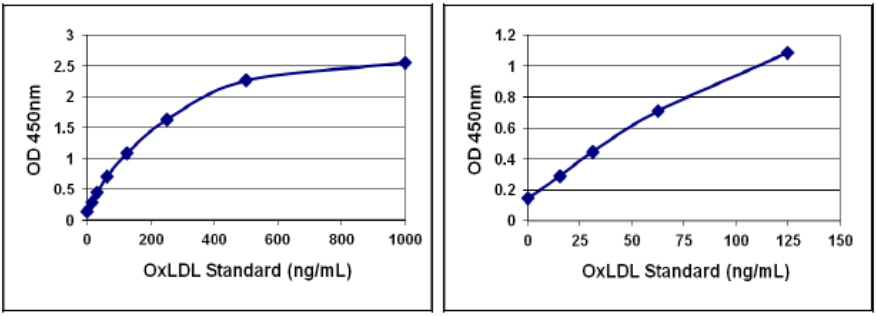

Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

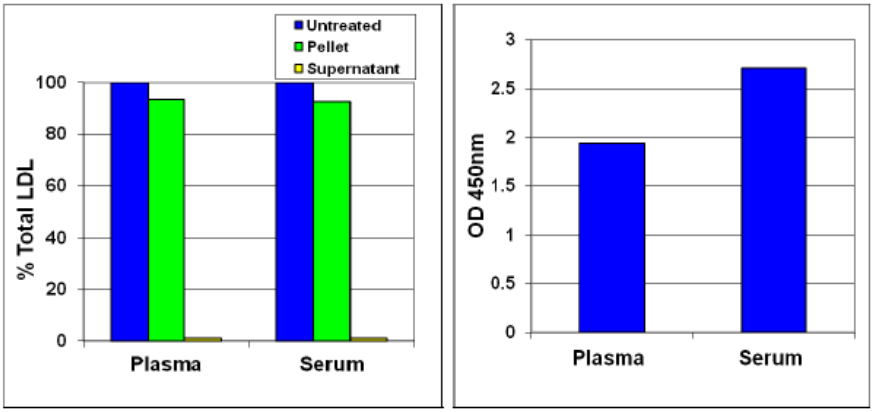

Figure 2. Quantitation of OxLDL in Serum and Plasma Samples.

Left: LDL Recovery After Precipitation Solution. Serum and plasma samples were treated with LDL Precipitation Solution according to the Sample Preparation protocol. LDL recovery was determined by ab242302.

Right: OxLDL Determination of Serum and Plasma Samples. Serum and plasma samples were treated with LDL Precipitation Solution according to the Sample Preparation Section. Precipitated LDL pellets were resuspended in 1.6 mL of PBS before further diluting 1:800 in Assay Diluent. Samples were tested according to the Assay Protocol.

12. Species and Cross Reactivity

This kit is not species specific and can be used with samples from any species.

Please contact our Technical Support team for more information.

13. Notes

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