Product Manual

Human LOX-1 ELISA Kit

Catalog Number

STA-387 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

The lipid-laden foam cells in atherosclerotic lesions represent monocyte/macrophages that have taken up lipoproteins in the subendothelial space. This is not the result of the uptake of native LDL, which cannot induce cholesterol accumulation in monocyte/macrophages, but rather it is due to the uptake of oxidized LDL (oxLDL). The lectin-like oxidized LDL receptor-1 (LOX-1) was identified as an oxLDL receptor expressed in vascular endothelium. It is also expressed in vascular smooth muscle cells, differentiated macrophages and platelets. LOX-1 is a 50 kDa type II transmembrane glycoprotein that structurally belongs to the C-type lectin family. It contains a short N-terminal cytoplasmic domain, a single transmembrane domain and an extracellular domain comprising a neck domain followed by a C-terminal C-type lectin-like domain. LOX-1 can be cleaved and released as a soluble form (sLOX-1). Serum levels of sLOX-1 are prognostic biomarkers of early acute coronary syndromes, stroke and coronary heart disease.

Cell Biolabs' Human LOX-1 ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human LOX-1 in plasma, serum or cell culture supernatant samples. The kit has a detection sensitivity limit of 40 pg/mL human LOX-1. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
2. STA-368: Human ApoB ELISA Kit
3. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
5. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human LOX-1 Antibody Coated Plate (Part No. 238701): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Human LOX-1 Antibody (500X) (Part No. 238702): One 25 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human LOX-1 Standard (Part No. 238703): One 50 µL vial of 1 µg/mL Human LOX-1 in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum or Cell Culture Supernatant
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human LOX-1 Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Human LOX-1 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Human LOX-1 antibody 1:500 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human LOX-1 Standard

Prepare a dilution series of human LOX-1 standards in the concentration range of 0 to 2500 pg/mL in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Human LOX-1 Standard (µL)	Assay Diluent (µL)	Human LOX-1 (pg/mL)
1	2	798	2500
2	400 of Tube #1	400	1250
3	400 of Tube #2	400	625
4	400 of Tube #3	400	313
5	400 of Tube #4	400	156
6	400 of Tube #5	400	78
7	400 of Tube #6	400	39
8	0	400	0

Table 1. Preparation of Human LOX-1 Standards

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell Culture Supernatant: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold RIPA buffer and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of human LOX-1 unknown sample or standard to the Anti-Human LOX-1 Antibody Coated Plate. Each human LOX-1 unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
3. 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.
4. Add 100 µL of the diluted Biotinylated Anti-Human LOX-1 Antibody to each well. Incubate at room temperature for 2 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. 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 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human LOX-1 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

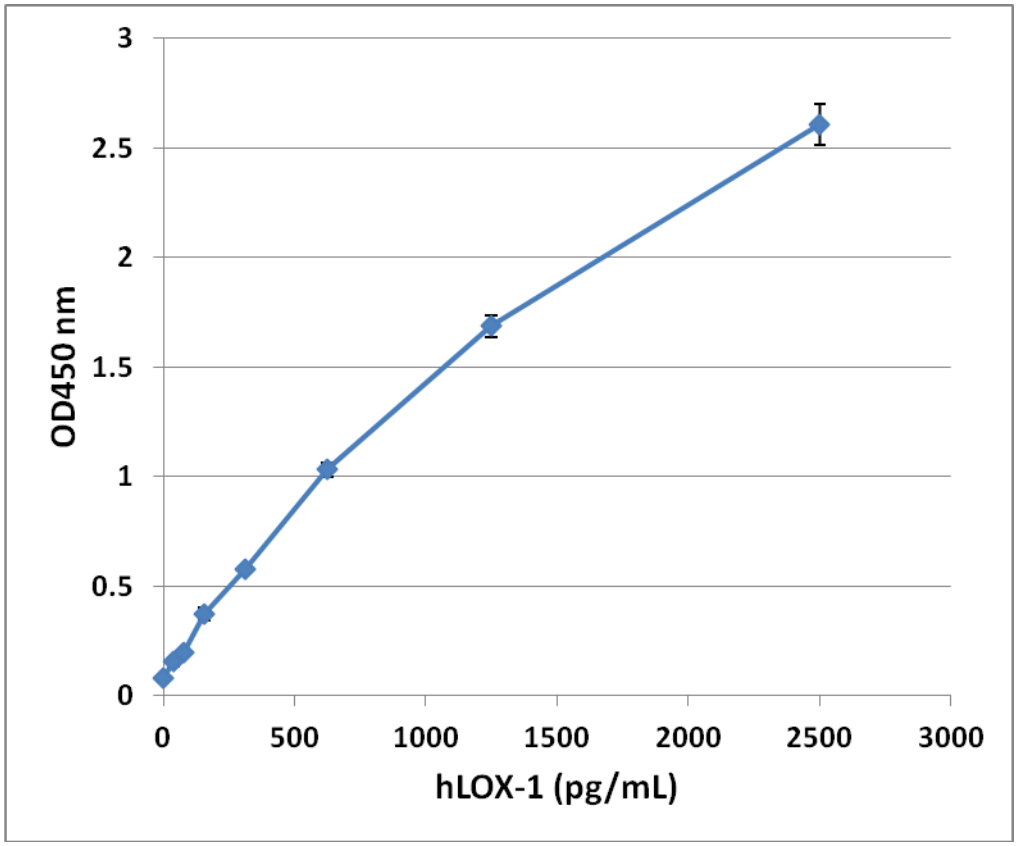

Figure 1: Human LOX-1 ELISA Standard Curve.

References

1. Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y, et al. *Nature.* 1997; 386:73–7.
2. Eto H, Miyata M, Kume N, Minami M, Itabe H, Orihara K, et al. *Biochem Biophys Res Commun.* 2006; 341:591–8.
3. Ishiyama J, Taguchi R, Yamamoto A, Murakami K. *Atherosclerosis.* 2010; 209:118–24.
4. Chen M, Kakutani M, Naruko T, Ueda M, Narumiya S, Masaki T, et al. *Biochem Biophys Res Commun.* 2001; 282:153–8.
5. Inoue N, Okamura T, Kokubo Y, Fujita Y, Sato Y, Nakanishi M, et al. *Clin Chem.* 2010; 56:550–8.

Recent Product Citations

1. Kook, H. et al. (2020). Identification of plaque ruptures using a novel discriminative model comprising biomarkers in patients with acute coronary syndrome. *Sci Rep*. **10**(1):20228. doi: 10.1038/s41598-020-77413-3.
2. Wu, J. et al. (2013). Clinical nephrology - IgA nephropathy, lupus nephritis, vasculitis. *Nephrol. Dial. Transplant*. **28**: i175-i184.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com