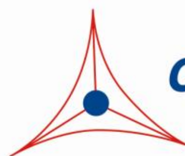
Product Manual

OxiSelect™ Human Oxidized HDL ELISA Kit (HNE-HDL Quantitation)

Catalog Number

STA-889 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



Introduction

Lipoproteins are submicroscopic particles composed of lipid and protein held together by noncovalent forces. Their general structure is that of a putative spheroidal microemulsion formed from an outer layer of phospholipids, unesterified cholesterol, and proteins, with a core of neutral lipids, predominately cholesteryl esters and triacylglycerols (TAG). High Density Lipoprotein (HDL) is a spherical particle with diameter of about 10 nm (Figure 1). HDL contains the Apoliprotein AI and AII molecules. HDL and LDL cholesterol levels in the blood are important indicators of many disease states. High blood levels of LDLs are associated with health problems and cardiovascular disease. For this reason, LDL is often referred to as the "bad cholesterol." LDL particles that accumulate within arteries can form plaques over time, which can increase chances of a stroke, heart attack, or vascular disease. HDL particles are able to remove cholesterol from within arteries and transport it back to the liver for re-utilization or excretion, which is the main reason why the cholesterol carried within HDL particles is sometimes called "good cholesterol" for its cardioprotective properties. Monitoring circulatory levels of different lipoproteins is critical to the diagnosis of lipid transport disorders such as atherosclerosis.

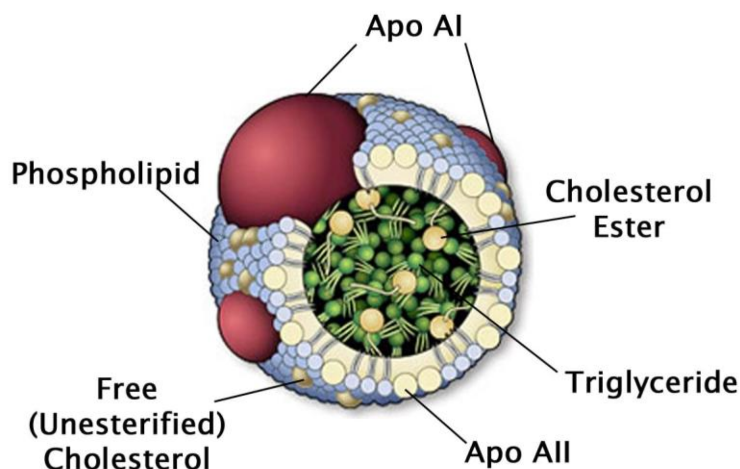


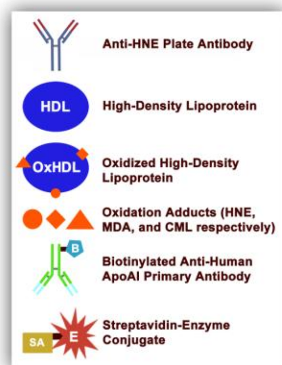
Figure 1: Structure of HDL.

When HDL becomes oxidized (OxHDL) it loses its cardioprotective properties and becomes dangerous to cells. HDL oxidation affects both its lipid and protein components: reactive aldehyde products, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), are formed during the oxidation of polyunsaturated fatty acids and are capable of attaching covalently to the ε-amino groups of lysine residues of Apo AI or Apo AII, forming MDA-Lys and HNE-Lys adducts (MDA-HDL and HNE-HDL). Oxidation of HDL causes a reduction in the ability of HDL to promote cellular cholesterol efflux through the ATP-binding cassette transporter A1 (ABCA1) pathway. Furthermore, treatment of monocytes and macrophages with OxHDL results in release of TNF alpha (a proinflammatory molecule), oxidative stress, and cytotoxicity. Similarly, OxHDL treatment of rat mesangial cells results in increased reactive oxygen species output, increased expression of TNF alpha as well as increased mesangial cell apoptosis.

The OxiSelect™ Human Oxidized HDL ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human OxHDL in plasma or serum. The kit contains an HNE-HDL

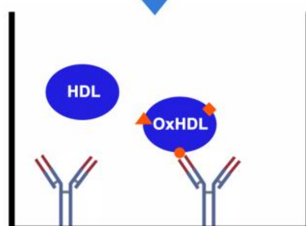
standard and has a detection sensitivity limit of 2 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Assay Principle

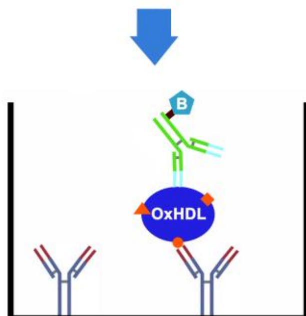


Human Plasma or Serum Sample

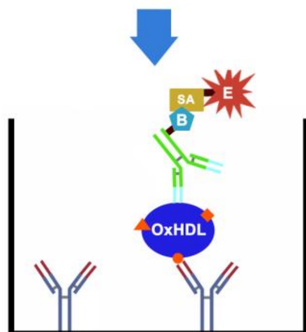
HDL is isolated accoridng to "Preparation of Samples" section



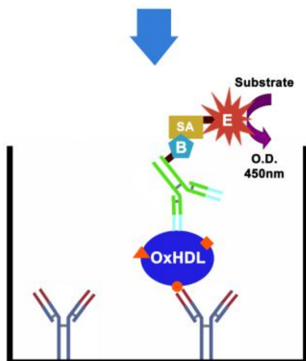
OxHDL is captured by the Anti-HNE Antibody Coated Plate



Captured OxHDL is incubated with Biotinylated Anti-Human ApoAI Primary Antibody



Streptavidin-Enzyme Conjugate is added for detection



Substrate is added and read on a microplate reader

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Related Products

1. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
2. STA-243: Human High Density Lipoprotein (HDL)
3. STA-358: Human Oxidized LDL ELISA Kit (OxPL-LDL Quantitation)
4. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
5. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)
6. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-HNE Antibody Coated Plate (Part No. 238901): One 96-well strip plate.
2. Biotinylated Anti-Human ApoAI Antibody (1000X) (Part No. 236202): One 20 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.
8. Precipitation Solution 1 (Part. No. 286901): One 250 µL vial.
9. Precipitation Solution 2 (Part. No. 286902): One 2.5 mL bottle.
10. Precipitation Solution 3 (Part. No. 286903): One 650 µL vial.
11. Buffer Solution (Part. No. 286904): One 5 mL bottle.
12. 5X Wash Solution (Part. No. 286905): One 1.2 mL vial.

Box 2 (shipped on blue ice packs)

1. Blocking Reagent (100X) (Part No. 238902): One 200 µL vial.
2. HNE-HDL Standard (Part No. 288901): One 20 µL vial of 0.5 mg/mL Human HNE-HDL.

Materials Not Supplied

1. Human Plasma or Serum Samples
2. PBS
3. Microcentrifuge
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips

6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Blocking Reagent and the HNE-HDL Standard at -80ºC. Avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Blocking Reagent: Immediately before use dilute the Blocking Reagent 1:100 with PBS. Do not store diluted solutions.
- Biotinylated Anti-Human ApoAI Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Anti-ApoAI antibody 1:1000 and Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.
- Resuspension Buffer: Dilute Precipitation Solution 3 1:100 and Precipitation Solution 2 1:10 in Buffer Solution. For example, add 1 uL of Precipitation Solution 3 and 10 uL of Precipitation Solution 2 to 89 uL of Buffer Solution. Mix well. Prepare only enough for immediate use and do not store unused buffer.
- 1X Wash Solution: Dilute the 5X Wash Solution to 1X with deionized water. Mix well. Store unused solution at 4ºC.

Preparation of HNE-HDL Standard

Dilute the 0.5 mg/mL HNE-HDL Standard 1:100 to 5 µg/mL in Assay Diluent. For example, add 4 µL of 0.5 mg/mL HNE-HDL Standard to 396 µL of Assay Diluent. Prepare a dilution series of HNE-HDL Standards in the concentration range of 0 to 80 ng/mL in Assay Diluent (Table 1).

Standard Tubes	5 µg/mL HNE-HDL Standard (µL)	Assay Diluent (µL)	Final HNE-HDL Standard (ng/mL)
1	16	984	80
2	250 of Tube #1	250	40
3	250 of Tube #2	250	20
4	250 of Tube #3	250	10
5	250 of Tube #4	250	5
6	250 of Tube #5	250	2.5
7	250 of Tube #6	250	1.25
8	0	250	0

Table 1. Preparation of HNE-HDL Standards

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Preparation of Samples

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

- Plasma or Serum: For preparation of plasma, collect blood with heparin or EDTA, centrifuge for 10 minutes at 1000 x *g* at 4ºC, and isolate plasma. For serum, harvest serum and centrifuge for 10 minutes at 1000 x *g* at 4ºC. Transfer 200 µL of plasma or serum to an eppendorf tube, add 5 µL of Precipitation Solution 1, and add 10 µL of Precipitation Solution 2, mixing well. Incubate at room temperature for 5 minutes (precipitation will occur). Centrifuge for 10 minutes at 6000 x *g* at 4ºC (pellet should be visible). Carefully collect the supernatant and transfer to a new eppendorf tube. Add 12 µL of Precipitation Solution 3 and 30 µL of Precipitation Solution 2, mixing well. Incubate at room temperature for 2 hours. Centrifuge for 30 minutes at 18-20000 x *g* at 4ºC (pellet should be visible). Discard the supernatant and resuspend pellet in 100 µL resuspension buffer. Mix thoroughly by pipetting up and down. Centrifuge for 10 minutes at 6000 x *g* at 4ºC. Discard supernatant and resuspend pellet in 120 µL of 1X Wash solution. Shake tube for 30 minutes at 4ºC. Shaking speed should be sufficient to dissolve pellet, but not so vigorous that bubbles form. Centrifuge tube again for 10 minutes at 6000 x *g* at 4ºC. Transfer the supernatant to a new tube and store at 4ºC if running the ELISA on the same day; otherwise store at -80C for up to 2 months. Further dilute the sample 1:50 to 1:200 in Assay Diluent before running the ELISA. Assay immediately and do not store solutions.

Assay Protocol

1. Please refer to the above Sample Preparation Section. Crude samples require the isolation steps described above prior to running the assay.
2. Add 100 µL of HNE-HDL standard or unknown sample to the Anti-HNE Antibody Coated Plate. Each HNE-HDL standard, blank and purified unknown sample should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 2 hours on an orbital shaker.
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.
5. Add 100 µL of diluted Blocking Reagent to each well. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
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.
7. Add 100 µL of the diluted Biotinylated Anti-Human Apo AI antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.

8. Wash the strip wells 5 times according to step 6 above.
9. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
10. Wash the strip wells 5 times according to step 6 above. Proceed immediately to the next step.
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 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. 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).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Example of Results

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

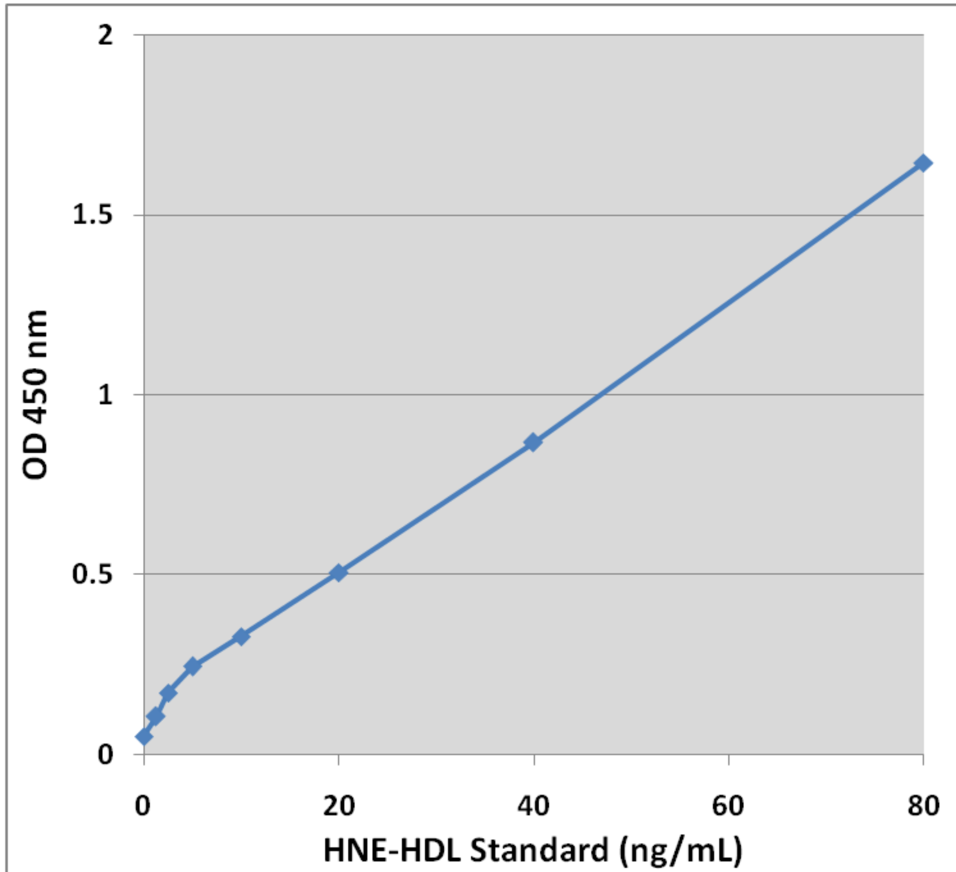


Figure 2: Human HNE-HDL Standard Curve.

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

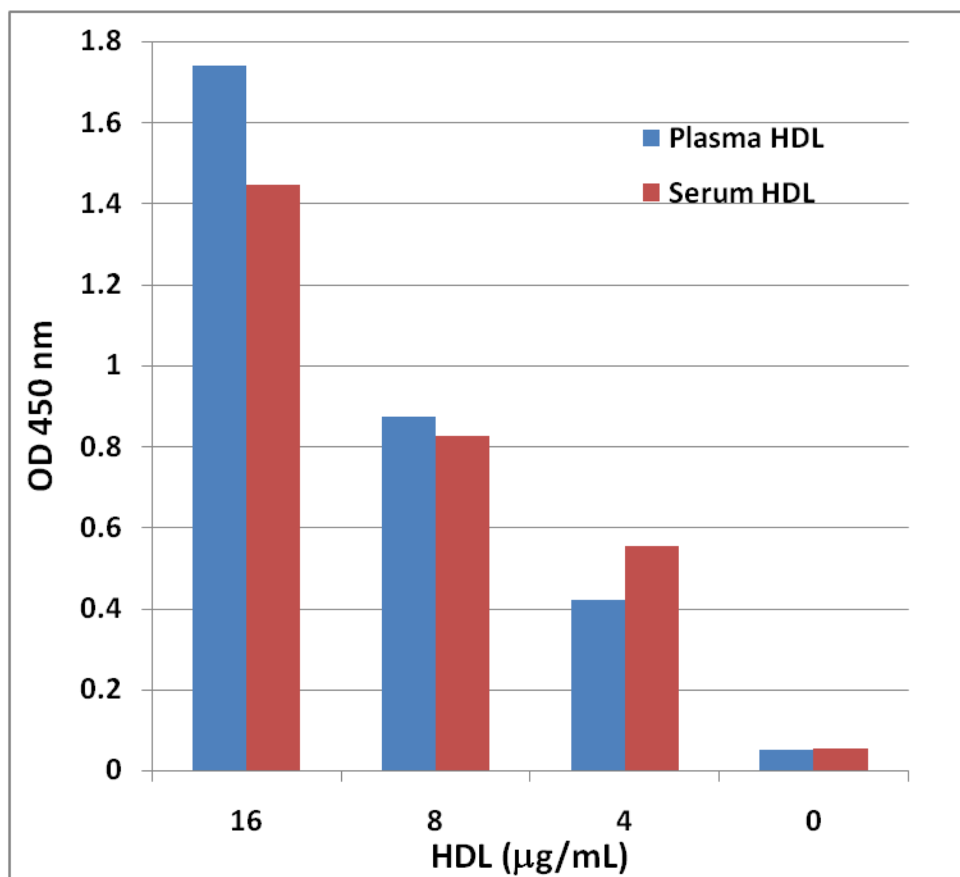


Figure 3: HNE-HDL Determination of Serum and Plasma Samples. HDL from human serum and plasma samples was isolated according to the Sample Preparation Section. Samples were diluted from 5 mg/mL to 16 µg/mL in Assay Diluent, and then twofold serial dilutions were prepared. Samples were tested according to the Assay Protocol.

References

1. Shao B. (2012) *Biochim Biophys Acta*. **1821**: 490-501.
2. Keeney J.T.R., Swomley A.M., Forster S., Harris J. L., Sultana R., and Butterfield D. A. (2013) *Proteomics Clin Appl.* **7**: 109-122.
3. Soumyarani V.S. and Jayakumari N. (2012) *Mol Cell Biochem.* **366**:277-285.
4. Zhang M., Gao X., Wu J., Liu D., Cai H., Fu L., and Mei C. (2010) 26:455-463
5. Smith C.K., Vivekanandan-Giri A., Tang C., Knight J.S., Mathew A., Padilla R.L., Gillespie B.W., Carmona-Rivera C., Liu X., Subramanian V., Hasni S., Thompson P.R., Heinecke J.W., Saran R., Pennathur S., and Kaplan M.J. (2014) *Arthritis Rheumatol*. **66**:2532-2544.

Recent Product Citation

Janac, J.M. et al. (2019). Increased Oxidized High-Density Lipoprotein/High-Density Lipoprotein-Cholesterol Ratio as a Potential Indicator of Disturbed Metabolic Health in Overweight and Obese Individuals. *Lab Med*. pii: lmz017. doi: 10.1093/labmed/lmz017.

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

©2014-2020: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.