Product Manual

LDL/VLDL and HDL Purification Kit (Ultracentrifugation Free)

Catalog Number

STA-608 **10 preps**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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). Very low-density lipoprotein (VLDL), a spherical particle with a diameter of 30-100 nm, is the major plasma vehicle for TAG and is the precursor to Low density lipoprotein (LDL). Each VLDL contains one molecule of a hydrophobic protein known as apolipoprotein B-100 (Apo B), as well as multiple copies of apolipoprotein E and apolipoprotein C (Figure 1 left).

LDL is the major transport protein for cholesterol in human plasma. LDL, like VLDL, is also a spherical particle with a diameter of 20-25 nm. Each LDL particle contains cholesteryl esters in its core which are surrounded by a hydrophilic coat composed of phospholipids, cholesterol, and one molecule apolipoprotein B-100 (Figure 1 right).

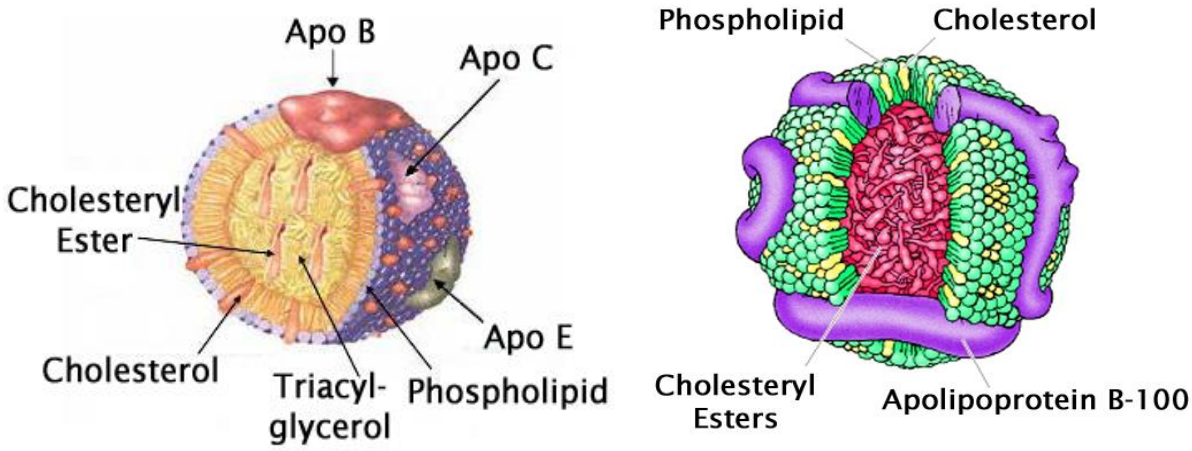

Figure 1: Structure of VLDL (left) and LDL (right).

High Density Lipoprotein (HDL) is also a spherical particle with diameter of about 10 nm (Figure 2). 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." Monitoring circulatory levels of different lipoproteins is critical to the diagnosis of lipid transport disorders such as atherosclerosis.

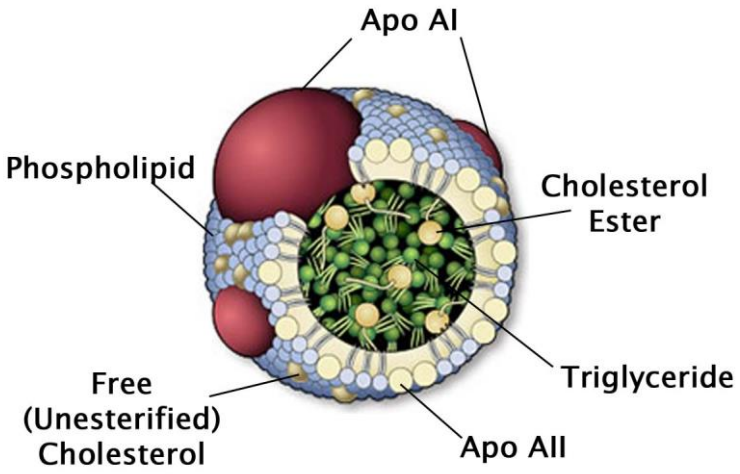

Figure 2: Structure of HDL.

The LDL/VLDL and HDL Purification Kit uses Dextran Sulfate to selectively and separately precipitate LDL/VLDL and HDL fractions. The kit allows for the purification of LDL/VLDL and/or HDL without the need for ultracentrifugation. The various lipoprotein particles are highly purified through a series of precipitation and low speed centrifugation steps. Each kit provides sufficient reagents to perform up to 10 preps, and each preparation can purify up to 10 mL of serum or plasma samples with a yield of ~600 µg of LDL/VLDL per mL and ~4500 µg of HDL per mL for human samples (expected yield will vary by species).

Related Products

1. STA-212: Malondialdehyde (MDA) Modified Human Low Density Lipoprotein (LDL)
2. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
3. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)
5. STA-390: Total Cholesterol Assay Kit (Fluorometric)
6. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
7. STA-606: LDL/VLDL Purification Kit (Ultracentrifugation Free)
8. STA-607: HDL Purification Kit (Ultracentrifugation Free)

Kit Components

1. Dextran Solution (Part No. 260801): One 8 mL bottle
2. Precipitation Solution A (Part No. 260802): One 30 mL amber bottle
3. Bicarbonate Solution (Part No. 260803): One 4 mL bottle
4. 10X Precipitation Solution B (Part No. 260804): One 10 mL bottle
5. Tris Solution (Part No. 260805): One 50 mL bottle containing 20 mM Tris, pH 7.5
6. NaCl Solution (Part No. 260806): One 6 mL bottle containing 5% NaCl
7. 10X Precipitation Solution C (Part No 260807): One 20 mL bottle
8. Dextran Removal Solution (Part No. 260808): One 10 mL bottle
9. 5X HDL Wash Solution (Part No. 260809): One 12 mL bottle

Materials Not Supplied

1. Serum or Plasma Samples
2. PBS
3. Microcentrifuge or Centrifuge
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips

Storage

Upon receipt store Dextran Removal Solution at room temperature. Store all other components at 4ºC.

Preparation of Reagents

- 1X Precipitation Solution B: Dilute the 10X Precipitation Solution B to 1X with deionized water. Stir to homogeneity. Store unused solution at 4ºC.
- 1X Precipitation Solution C: Dilute the 10X Precipitation Solution C to 1X with deionized water. Stir to homogeneity. Store unused solution at 4ºC.
- HDL Resuspension Buffer: Dilute Dextran Solution 1:100 and Precipitation Solution A 1:10 in Tris Solution. For example, add 50 µL of Dextran Solution and 0.5 mL of Precipitation Solution A to 4.45 mL of Tris Solution. Stir to homogeneity. Prepare only enough for immediate use and do not store unused buffer.
- 1X HDL Wash Solution: Dilute the 5X HDL Wash Solution to 1X with deionized water. Stir to homogeneity. Store unused solution at 4ºC.

Purification Protocol

Note: The purification protocol below is written for a 10 mL sample size. For smaller sample volumes, scale down each step proportionally.

I. Dextran Precipitation

1. To 10 mL of serum or plasma on ice, add 50 µL of Dextran Solution and 500 µL of Precipitation Solution A. Incubate 5 minutes on ice.
2. Spin at 6000 x g 10 minutes at 4ºC.
3. Remove the supernatant, which contains HDL, for use in section III below. Use the remaining pellet which contains LDL and VLDL for section II below.

   Note: The HDL-containing supernatant may be stored at 4ºC for future processing.

II. LDL/VLDL Purification

1. Resuspend the pellet from section I above with 400 µL of Bicarbonate Solution and spin at 6000 x g 10 minutes at 4ºC.
2. Transfer the supernatant to a new tube. Discard the pellet.
3. Add 10 mL of 1X Precipitation Solution B to the supernatant. Mix thoroughly by pipetting up and down.
4. Spin at 6000 x g for 10 minutes at 4ºC.
5. Discard the supernatant and resuspend the pellet with 200 µL of NaCl Solution.
6. Add 10 mL of 1X Precipitation Solution C. Mix thoroughly by pipetting up and down.
7. Spin at 6000 x g for 10 minutes at 4ºC.
8. Repeat steps 5-7.
9. Resuspend the pellet in 200 µL of NaCl Solution (final volume about 500 µL).
10. Add 80 µL of Dextran Removal Solution. Mix thoroughly by pipetting up and down and incubate 1 hour at 4ºC.
11. Spin at 6000 x g for 10 minutes at 4ºC.
12. Recover the supernatant (purified LDL/VLDL) and transfer to a new tube.
13. Dialyze the purified LDL/VLDL in PBS and determine the protein concentration.

III. HDL Purification

1. To 10 mL of supernatant from section I above, add 600 µL of Dextran Solution and 1.5 mL of Precipitation Solution A. Incubate for 2 hours at room temperature.
2. Spin 18,000-20,000 x g for 30 minutes at 4ºC.
3. Discard supernatant and resuspend pellet in 5 mL of HDL Resuspension Buffer (see Preparation of Reagents Section). Mix thoroughly by pipetting up and down.

4. Spin 6000 x g for 10 minutes at 4ºC.
5. Discard supernatant and resuspend pellet in 6 mL of 1X HDL Wash Solution (see Preparation of Reagents Section).
6. Shake for 30 minutes at 4ºC. Shaking speed should be sufficient to dissolve pellet, but not so vigorous that bubbles form.
7. Spin 6000 x g for 10 minutes at 4ºC.
8. Transfer the supernatant to a new tube and add 900 µL of Dextran Removal Solution. Mix thoroughly by pipetting up and down.
9. Incubate for 1 hour at 4ºC.
10. Spin 6000 x g for 10 minutes at 4ºC.
11. Transfer the supernatant (containing purified HDL) to a new tube.
12. Dialyze the purified HDL in PBS and determine the protein concentration.

Example of Results

The following figures demonstrate typical results with the LDL/VLDL and HDL Purification Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

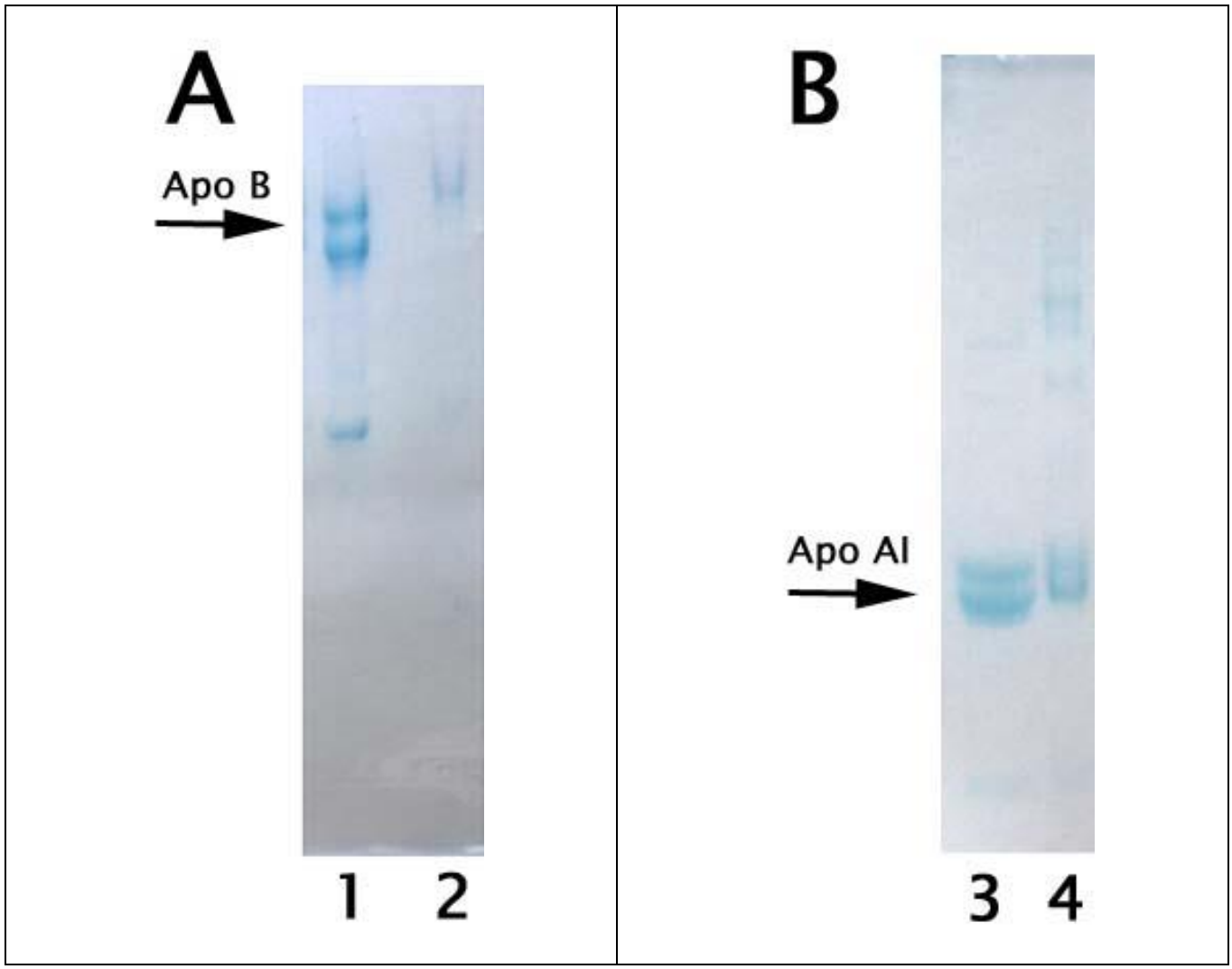

Figure 3: SDS PAGE gels of purified Lipoproteins. 20 µg of LDL/VLDL (A) or HDL (B) purified by the STA-608 kit (lanes 1 and 3) or ultracentrifugation (lanes 2 and 4) were loaded on a 3-8% Tris Acetate Gel (A) or a 12% Bis Tris Gel (B) and stained with Coomassie Brilliant Blue Dye.

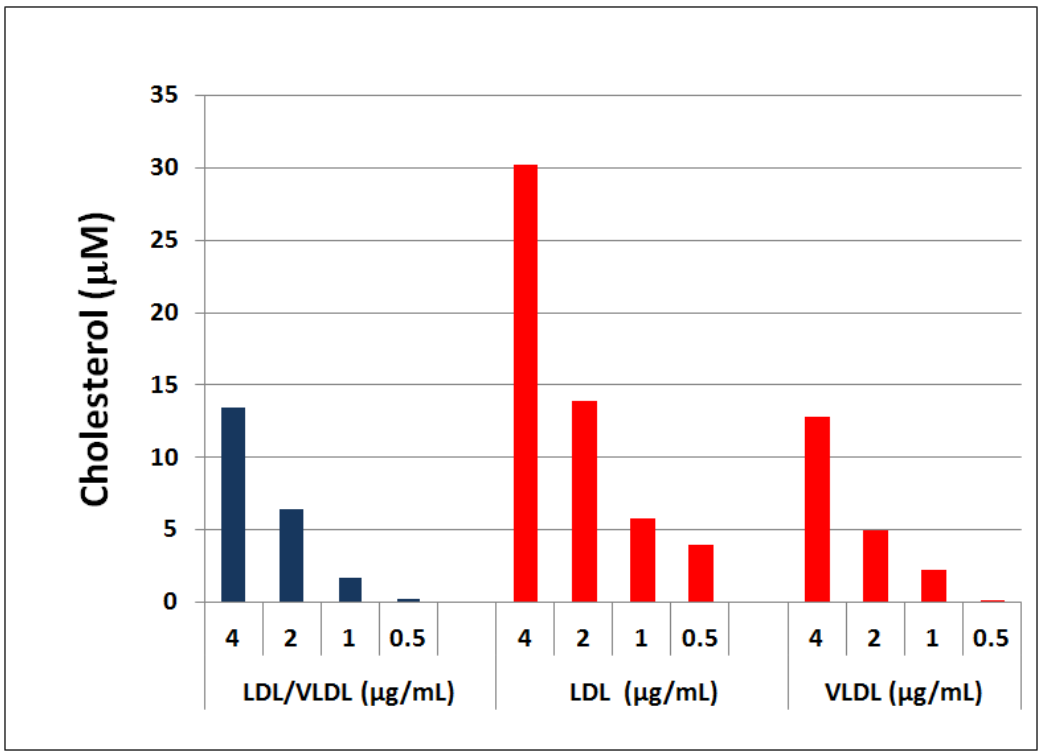

Figure 4: Detection of Cholesterol in purified LDL/VLDL Samples. Purified LDL/VLDL isolated using the LDL/VLDL and HDL Purification Kit (blue bars) or ultracentrifugation (red bars) was tested for the presence of Cholesterol using the Total Cholesterol Assay Kit (Cat. # STA-390).

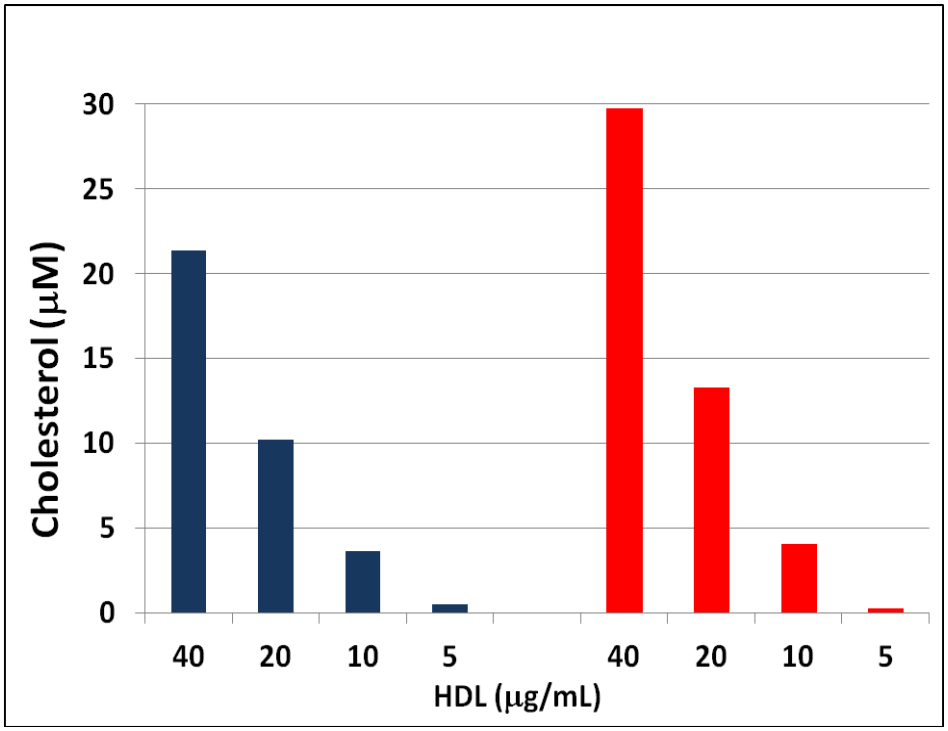

Figure 5: Detection of Cholesterol in purified HDL Samples. Purified HDL from the LDL/VLDL and HDL Purification Kit (blue bars) or ultracentrifugation (red bars) was tested for the presence of Cholesterol using the Total Cholesterol Assay Kit (Cat. # STA-390).

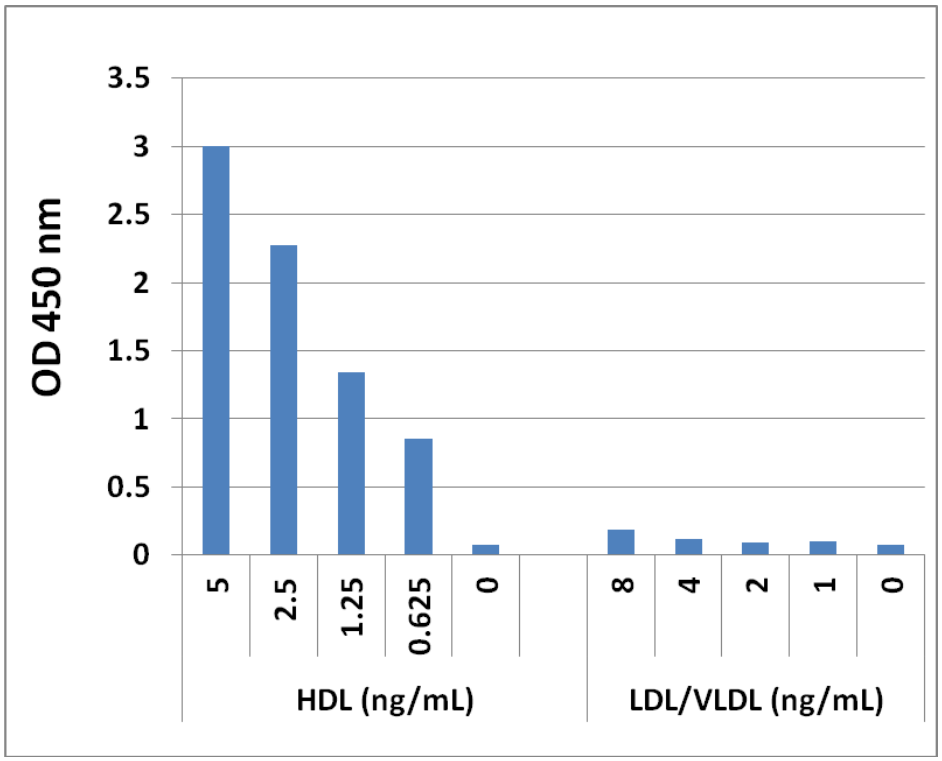

Figure 6: Detection of ApoA1 in purified Lipoprotein Samples. Purified HDL or LDL/VLDL from the LDL/VLDL and HDL Purification Kit was tested for the presence of ApoA1 by ELISA.

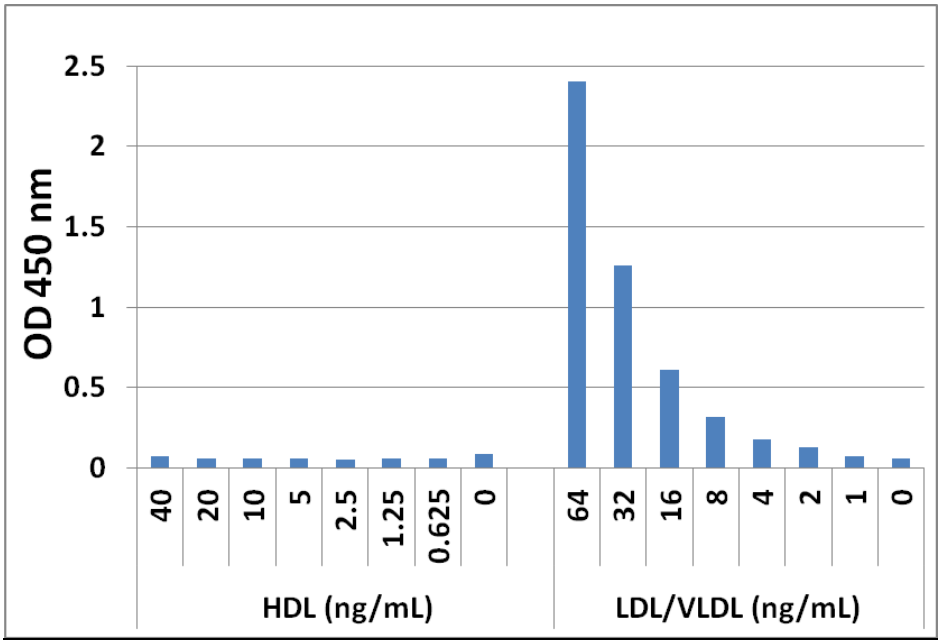

Figure 7: Detection of ApoB in purified Lipoprotein Samples. Purified HDL or LDL/VLDL from the LDL/VLDL and HDL Purification Kit was tested for the presence of ApoB by ELISA.

References

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Recent Product Citations

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