Datasheet for ABIN2345117

LDL/VLDL Purification Kit (Ultracentrifugation Free)

1 Publication

Go to Product page

Overview

Quantity:	10 preparations
Application:	Purification (Purif)

Product Details

Purpose:	The LDL/VLDL Purification Kit uses Dextran Sulfate to selectively precipitate LDL/VLDL from plasma or serum.
Sample Type:	Plasma, Serum
Characteristics:	The kit allows for the purification of LDL/VLDL without the need for ultracentrifugation. The 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 for human samples (expected yield will vary by species).
Components:	1. Dextran Solution : One 0.6 mL vial 2. Precipitation Solution A : One 6 mL amber bottle 3. Bicarbonate Solution : One 4 mL bottle 4. 10X Precipitation Solution B : One 10 mL bottle 5. NaCl Solution : One 6 mL bottle containing 5% NaCl 6. 10X Precipitation Solution C : One 20 mL bottle 7. Dextran Removal Solution : One 1.6 mL vial
Material not included:	1. Serum or Plasma Samples 2. PBS 3. Microcentrifuge or Centrifuge 4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips

Target Details

Background:	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).

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Reagent Preparation:	• 1X Precipitation Solution B: Dilute the 10X Precipitation Solution B to 1X with deionized water. Stir to homogeneity. Store unused solution at 4 °C. • 1X Precipitation Solution C: Dilute the 10X Precipitation Solution C to 1X with deionized water. Stir to homogeneity. Store unused solution at 4 °C. 3 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 4 °C. 3. Discard the supernatant. Use the remaining pellet which contains LDL and VLDL for section II below. 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 4 °C. 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 4 °C. 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 4 °C. 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 4 °C. 11. Spin at 6000 x g for 10 minutes at 4 °C. 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. 4
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Upon receipt store Dextran Removal Solution at room temperature. Store all other components at 4°C.

Publications

Product cited in:	Ito, Ito, Suzuki, Yahata, Ikeda, Hamaoka: "The Application of a Modified d-ROMs Test for Measurement of Oxidative Stress and Oxidized High-Density Lipoprotein." in: **International journal of molecular sciences**, Vol. 18, Issue 2, (2017) (PubMed).