HDL and LDL / VLDL Assay Kit

HDL and LDL / VLDL Assay Kit is a detection kit for the quantification of HDL and LDL / VLDL in serum.

Catalog number: ARG81629

Package: 100 tests

TABLE OF CONTENTS

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INTRODUCTION

A lipoprotein is a biochemical assembly whose primary function is to transport hydrophobic lipid (also known as fat) molecules in water, as in blood plasma or other extracellular fluids. They consist of a Triglyceride and Cholesterol center, surrounded by a phospholipid outer shell, with the hydrophilic portions oriented outward toward the surrounding water and lipophilic portions oriented inward toward the lipid center. A special kind of protein, called apolipoprotein, is embedded in the outer shell, both stabilising the complex and giving it a functional identity that determines its role.

Many enzymes, transporters, structural proteins, antigens, adhesins and toxins are lipoproteins. Examples include plasma lipoprotein particles (HDL, LDL, IDL, VLDL and chylomicrons). Subgroups of these plasma particles are primary drivers or modulators of atherosclerosis. [Provide by Wikipedia: Lipoprotein]

PRINCIPLE OF THE ASSAY

This HDL and LDL / VLDL Assay Kit is a simple colorimetric assay that measures the concentrations of HDL and LDL / VLDL in serum samples. This kit is based on our improved PEG precipitation method in which HDL and LDL/VLDL are separated, and cholesterol concentrations are determined using cholesterol esterase / cholesterol dehydrogenase reagent. In this reaction, NAD is reduced to NADH. The optical density of the formed NADH at O.D. 340 nm is directly proportionate to the cholesterol concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	20 mL	-20°C
PBS	1.5 mL	-20°C
Precipitation Reagent	1.5 mL	-20°C
Enzyme Mix	120 µL	-20°C
NAD Solution	2 mL	-20°C
Standard (300 mg/dL cholesterol)	10 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 340 nm
- Clear flat-bottom 96 well microplate
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Transfer 20 µL of serum sample into a 1.5 mL centrifuge tube, add 20 µL of Precipitation Reagent. Vortex to mix and centrifuge for 5 minutes at 9,500 x g. Use the treated sample for HDL group and LDL / VLDL group as follow.

HDL group: Carefully transfer 24 µL of supernatant into a clean tube, add 96 µL of Assay Buffer. Label this tube "HDL".

LDL / VLDL group: Carefully remove all remaining supernatant from the pellet. Transfer 40 µL of PBS to the pellet and mix by repeated pipetting. Transfer 24 µL of mixture into another clean tube, add 96 µL of Assay Buffer. Label this tube "LDL / VLDL".

Total group: transfer 12 µL of serum sample and mix well with 108 µL of Assay Buffer. Label this tube "Total".

Note:

- Do not use haemolytic serum samples.

REAGENT PREPARATION

- **Working Reagent:** for each well, mixing 50 µL of Assay Buffer, 18 µL of NAD Solution and 1 µL of Enzyme Mix. Fresh reconstitution is recommended.
- **Working Standard:** transfer 12 µL of Standard (300 mg/dL cholesterol) and mix with 108 µL of Assay Buffer.

ASSAY PROCEDURE

Equilibrate all components except enzyme mix to room temperature. Briefly centrifuge tubes before use. The following procedure is designed for duplicate determinations.

	Blank well	Standard well	Total well	HDL well	LDL/VLDL well
Working Standard		50 µL			
Assay Buffer	50 µL				
HDL group				50 µL	
LDL/VLDL group					50 µL
Total group			50 µL		
Working Reagent	60 µL	60 µL	60 µL	60 µL	60 µL
Tap plate to mix briefly. Incubate for **30 minutes** at **room temperature**.					
Read the absorbance at **O.D. 340 nm**.					

Note: addition of Working Reagent to all wells should be rapid and mixing should be thorough. Use of a multichannel pipettor is recommended.

CALCULATION OF RESULTS

1. Cholesterol concentrations in the Total, HDL and (LDL/VLDL) fractions are calculated as follows:

   Total (mg/dL) = $[(OD_{Total} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] \times 300$

   HDL (mg/dL) = $[(OD_{HDL} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] \times 300$

   LDL/VLDL (mg/dL) = $[(OD_{LDL/VLDL} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] \times 300$

   Note:

   OD_{Total}, OD_{HDL}, $OD_{LDL/VLDL}$, $OD_{Standard}$ and OD_{Blank}: the O.D. 340 nm value of Total, HDL, LDL/VLDL group sample, Standard and Blank (Assay Buffer).

QUALITY ASSURANCE

Sensitivity

5 mg/dL