

User's Manual and Instructions

HDL and LDL/VLDL Assay Kit (Z5030057)

Quantitative Colorimetric Determination of HDL and LDL/VLDL Cholesterol

DESCRIPTION

CHOLESTEROL concentrations in *High-Density Lipoprotein (HDL)* and *Low-Density (LDL)/Very-Low-Density (VLDL) Lipoproteins* are strong predictors for coronary heart disease. Functional HDL offers protection by removing cholesterol from cells and atheroma. Higher concentrations of LDL and lower concentrations of functional HDL are strongly associated with cardiovascular disease due to higher risk of atherosclerosis. The balances between high- and low-density lipoproteins are solely genetically determined, but can be changed by medications, food choices and other factors.

Simple, direct and automation-ready procedures for measuring HDL and LDL/VLDL concentrations are very desirable. BioChain's HDL and LDL/VLDL quantification 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 340 nm is directly proportionate to the cholesterol concentration in the sample.

APPLICATIONS

Direct Assays: HDL and LDL/VLDL cholesterol in serum samples from any species.

Pharmacology: evaluation of drugs on cholesterol metabolism.

KEY FEATURES

Sensitive and accurate. Requires only 20 μ L serum sample. Detection limit of 5 mg/dL, linearity up to 300 mg/dL cholesterol in 96-well plate assay.

Convenient. Room temperature assay. No 37°C heater is needed.

KIT CONTENTS (100 assays in 96-well plates)

PBS: 1.5 mL **Precipitation Reagent: 1.5 mL**
Assay Buffer: 20 mL **Enzyme Mix: 120 μ L**
NAD Solution: 2 mL **Standard: 1 mL 300mg/dL cholesterol**

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

Precautions: 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.

PROCEDURES

Important: bring all reagents except enzyme mix to room temperature prior to assay. Non-hemolyzed serum samples should be used. The following procedure is designed for duplicate determinations.

1. **Sample Preparation.** Transfer 20 μ L serum into a 1.5-mL centrifuge tube, add 20 μ L Precipitation Reagent. Vortex to mix and centrifuge 5 min at 9,500 x g (e.g. 9,500 rpm in an Eppendorf 5415C tabletop centrifuge).

Carefully transfer 24 μ L supernatant into a clean tube, add 96 μ L Assay Buffer. Label this tube "HDL".

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

In a third tube, transfer 12 μ L serum sample and mix well with 108 μ L Assay Buffer. Label this tube "Total".

Cholesterol Standard: transfer 12 μ L 300 mg/dL cholesterol and mix with 108 μ L Assay Buffer. Label this tube "Standard".

2. **Assay.** Transfer 50 μ L Assay Buffer ("Blank"), 50 μ L Standard, 50 μ L "Total", 50 μ L "HDL" and 50 μ L "LDL/VLDL" into wells of a clear bottom 96-well plate. If desired, run assays in duplicate.

Prepare enough Working Reagent. For each reaction well, mix 50 μ L Assay Buffer, 18 μ L NAD Solution and 1 μ L Enzyme Mix.

Transfer 60 μ L of the Working Reagent to each reaction well. Tap plate to mix well. *Note: addition of Working Reagent to all wells should be rapid and mixing should be thorough. Use of a multi-channel pipettor is recommended.*

Incubate 30 min at room temperature. Read OD values at 340nm.

3. **Calculation.** Cholesterol concentrations in the Total, HDL and (LDL/VLDL) fractions are calculated as follows,

$$[\text{Total}] = \frac{\text{OD}_{\text{TOTAL}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{BLANK}}} \times 300 \text{ (mg/dL)}$$

$$[\text{HDL}] = \frac{\text{OD}_{\text{HDL}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{BLANK}}} \times 300 \text{ (mg/dL)}$$

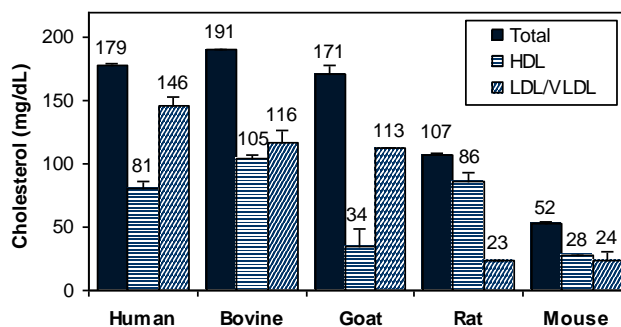
$$[\text{LDL/VLDL}] = \frac{\text{OD}_{\text{LDL/VLDL}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{BLANK}}} \times 300 \text{ (mg/dL)}$$

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting (multi-channel) devices, clear bottom 96-well plate and plate reader.

EXAMPLES

Serum samples were run in duplicate according to the standard procedure.



LITERATURE

[1]. Viikari J. (1976). Precipitation of plasma lipoproteins by PEG 6000 and its evaluation with electrophoresis and ultracentrifugation. Scand J Clin Lab Invest 36:265-268.

[2]. Demacker PMN, Humans AGM, Vos-Janssen HE, van't Laar A, Jansen AP. (1980). A study of the use of polyethylene glycol in estimating cholesterol in high density lipoprotein. Clin Chem 26:1775-9.

[3]. Widhaim, K. and Pakosta, R. (1991). Precipitation with Polyethylene Glycol and Density-Gradient Ultracentrifugation Compared for Determining High-Density Lipoprotein Subclasses HDL2 and HDL3. Clin. Chem 37/2, 238-240.