



HDL and LDL/VLDL Assay Kit

Catalog Number KA1671

100 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

Application:

- ✓ Direct Assays: HDL and LDL/VLDL cholesterol in serum samples from any species.
- ✓ Pharmacology: evaluation of drugs on cholesterol metabolism.

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.

Principle of the Assay

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. HDL and LDL/VLDL Assay 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.

General Information

Materials Supplied

List of component

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

Storage Instruction

Store all components at -20°C.

Materials Required but Not Supplied

- ✓ Pipetting (multi-channel) devices
- ✓ Clear bottom 96-well plate
- ✓ Plate reader

Precautions for Use

- ✓ Precautions
Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Assay Procedure

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 μL serum into a 1.5-mL centrifuge tube, add 20 μL 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 μL supernatant into a clean tube, add 96 μL Assay Buffer. Label this tube "HDL".

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

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

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

2. **Assay.** Transfer 50 μL Assay Buffer ("Blank"), 50 μL Standard, 50 μL "Total", 50 μL "HDL" and 50 μL "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 μL Assay Buffer, 18 μL NAD Solution and 1 μL Enzyme Mix.

Transfer 60 μL 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 340 nm.

Data Analysis

Calculation of Results

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

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

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

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

Examples

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

