HDL and LDL/V LDL Assay Kit (Colorimetric)

LS-K316-100 (100 Tests) • **Store at -20°C**

Introduction

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. LSBio'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.

Key Features

- Sensitive and accurate. Requires only 20 µL 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.

Applications

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

Pharmacology: evaluation of drugs on cholesterol metabolism.

Components

Component	K316-100 100 Tests
PBS	1.5 mL
Assay Buffer	20 mL
NAD Solution	2 mL
Precipitation Reagent	1.5 mL
Enzyme Mix	120 µL
Standard (300mg/dL cholesterol)	1 mL

Materials Not Supplied

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

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

HDL and LDL/V LDL Assay Kit (Colorimetric)

LS-K316-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Assay Procedure

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 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", 50µL "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 multichannel pipette 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,

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

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

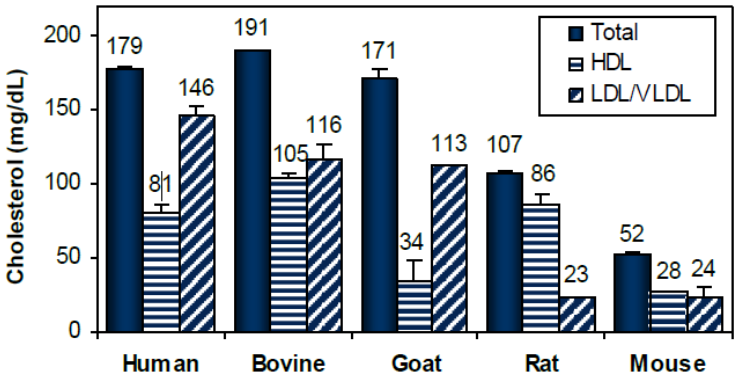
$$[\text{LDL/VLDL}] = \frac{OD_{LDL/VLDL} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 300 \ (\text{mg/dL})$$

HDL and LDL/V LDL Assay Kit (Colorimetric)

LS-K316-100 (100 Tests) • **Store at -20°C**

Sample Data

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



Version: V.08.09.2018