AF HDL and LDL /VLDL Assay Kit (Colorimetric/Fluorometric)

LS-K314-100 (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 a single Working Reagent that combines cholesterol ester hydrolysis, oxidation and color reaction in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λem/ex = 585/530nm is directly proportional to total cholesterol concentration in the sample.

Key Features

- Sensitive and accurate. Linear detection range in 96 -well plate: 1 to 100 mg/dL cholesterol for colorimetric assays and 0.2 to 1 0 mg/dL for fluorometric assays.
- Convenient. Room temperature assay. No 37°C heater is needed.

Applications

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

Components

Component	K314-100
	100 Tests
Developer	12 mL
Co-Substrate	120 µL
Dye Reagent	400 µL
Pyruvate Standard	25 mM

Materials Not Supplied

Pipetting devices, 96-well plate and plate reader.

Storage

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

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Assay Procedure

Important:

Equilibrate Developer to desired assay temperature.

Sample Preparation

Tissue or cell samples (2×10^6) can be homogenized in 100 µL PBS. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay. Serum should be diluted at least 4-fold in dH_2O.

Colorimetric Procedure

Important: bring all reagents except enzyme mix to room temperature prior to assay. Non-hemolyzed serum samples should be used.

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 5 µL 300 mg/dL cholesterol and mix with 145 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 flat-bottom 96-well plate. If desired, run assays in duplicate.

   For each reaction well, mix 55 µL Assay Buffer with 1 µL Enzyme Mix and 1 µL Dye Reagent. Add 50 µL of this Working Reagent to each standard and sample well. Tap plate to mix well. Incubate 30 min at room temperature. Read OD values at 570 nm.

   Note: if the Sample OD is higher than the Standard OD, dilute sample in assay buffer and repeat the assay. Multiply result by the dilution factor.

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

$$[\text{Total}] = \frac{OD_{TOTAL} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 100 \ (\text{mg/dL})$$

$$[\text{HDL}] = \frac{OD_{HDL} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 100 \ (\text{mg/dL})$$

$$[\text{LDL/VLDL}] = \frac{OD_{LDL/VLDL} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 100 \ (\text{mg/dL})$$

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Fluorometric Procedure

Dilute the Samples and Standard prepared in Colorimetric Procedure 1:10 in Assay Buffer. Transfer 50 µL diluted standards and 50 µL diluted samples into separate wells of a black 96-well plate.

Add 50 µL Working Reagent (see Colorimetric Procedure). Tap plate to mix. Incubate 30 min at room temperature and read fluorescence at λex = 530nm and λem = 585nm.

Note: if the Sample F is higher than the Standard F, dilute sample in assay buffer and repeat the assay. Multiply result by the dilution factor.

The cholesterol concentration of Sample is calculated as

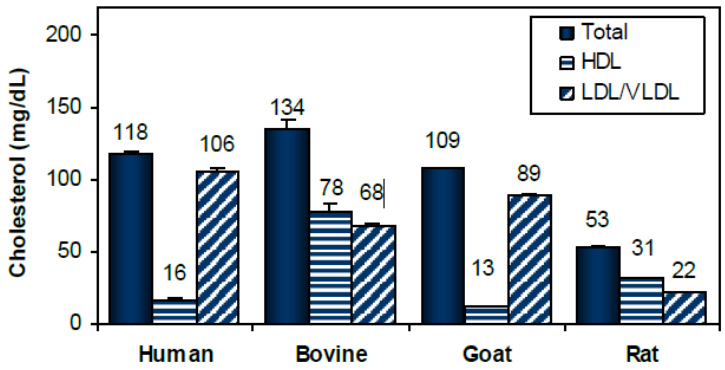
$$[\text{Total}] = \frac{F_{TOTAL} - F_{BLANK}}{F_{STANDARD} - F_{BLANK}} \times 100 \ (\text{mg/dL})$$

$$[\text{HDL}] = \frac{F_{HDL} - F_{BLANK}}{F_{STANDARD} - F_{BLANK}} \times 100 \ (\text{mg/dL})$$

$$[\text{LDL/VLDL}] = \frac{F_{LDL/VLDL} - F_{BLANK}}{F_{STANDARD} - F_{BLANK}} \times 100 \ (\text{mg/dL})$$

Sample Data

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



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