EnzyChrom™ HDL and LDL/VLDL Assay Kit (EHDL–100)
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. BioAssay Systems' 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 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.

KIT CONTENTS (100 assays in 96-well plates)
PBS: 1.5 mL   Precipitation Reagent: 1.5 mL
Assay Buffer: 20 mL   Enzyme Mix: 120 uL
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 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 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,

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

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

$$[\text{LDL/VLDL}] = \frac{OD_{LDL/VLDL} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 300 \ (\text{mg/dL})$$
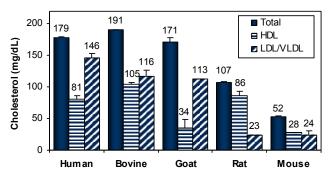
MATERIALS REQUIRED, BUT NOT PROVIDED
Pipetting (multi-channel) devices, clear bottom 96-well plate and plate reader.

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

PUBLICATIONS
1. Ponda, MP et al (2010). Moderate kidney disease inhibits atherosclerosis regression. Atherosclerosis 210 (1): 57-62.

2. Tam, J et al. (2010). Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. J Clin Invest. 120(8): 2953-2966.

3. Klusonova P et al (2011). Local metabolism of glucocorticoids in Prague hereditary hypertriglyceridemic rats - effect of hypertriglyceridemia and gender. Steroids 76(12):1252-1259.