EnzyChrom™ AF HDL and LDL/VLDL Assay Kit (E2HL-100)

Quantitative Colorimetric/Fluorimetric Determination of HDL and LDL/VLDL

DESCRIPTION

CHOLESTEROL concentrations in *H*igh-*D*ensity *L*ipoprotein (HDL) and *L*ow-*D*ensity (LDL)/*V*ery-*L*ow-*D*ensity (VLDL) *L*ipoproteins 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 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.

APPLICATIONS

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

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 10 mg/dL for fluorimetric assays.
Convenient. Room temperature assay. No 37°C heater is needed.

KIT CONTENTS (100 assays in 96-well plates)

PBS: 2 × 1.5 mL	**Precipitation Reagent: 1.5 mL**
Assay Buffer: 20 mL	**Enzyme Mix: 120 uL**
Dye Reagent: 120 µL	**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.

COLORMETRIC PROCEDURES

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,

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

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

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

FLUORIMETRIC 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 λ_{ex} = 530nm and λ_{em} = 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

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

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

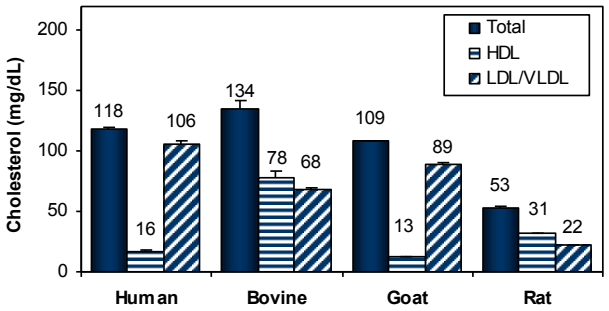
$$[\text{LDL/VLDL}] = \frac{F_{LDL/VLDL} - F_{BLANK}}{F_{STANDARD} - F_{BLANK}} \times 100 \text{ (mg/dL)}$$

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, 96-well plate and plate reader.

EXAMPLES

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



PUBLICATIONS

1. Wan W et al (2011). Genetic deletion of chemokine receptor Ccr6 decreases atherogenesis in ApoE-deficient mice. Circ Res. 109(4):374-381.

2. Uddin, MJ et al (2011). Detection of quantitative trait loci affecting serum cholesterol, LDL, HDL, and triglyceride in pigs. BMC Genetics 12:62.

3. Bourdon JA et al (2012) Hepatic and pulmonary toxicogenomic profiles in mice intratracheally instilled with carbon black nanoparticles reveal pulmonary inflammation, acute phase response, and alterations in lipid homeostasis. Toxicol Sci 127(2):474-484.