

Technical Manual

ColorFluor AF HDL and LDL/VLDL Assay Kit

Catalogue Code: BA0066

Pack Size: 100 assays

Research Use Only

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. The Assay Genie ColorFluor AF 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 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 $\lambda_{em/ex} = 585/530\text{nm}$ 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 μL

Dye Reagent: 120 μL **Standard:** 1 mL 300mg/dL cholesterol

Storage conditions. The kit is shipped on ice. Store PBS and Precipitation Reagent at room temperature and other reagents 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.

- 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 $\times g$ (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".
- 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 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,

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

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

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

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 $\lambda_{\text{ex}} = 530\text{nm}$ and $\lambda_{\text{em}} = 585\text{nm}$.

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

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

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

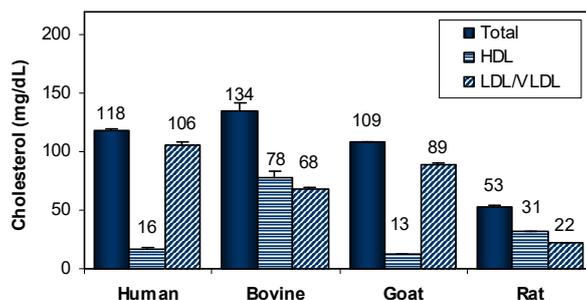
$$[\text{LDL/VLDL}] = \frac{F_{\text{LDL/VLDL}} - F_{\text{BLANK}}}{F_{\text{STANDARD}} - F_{\text{BLANK}}} \times 100 \text{ (mg/dL)}$$

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.

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