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Product Information

AF HDL and LDL/VLDL Assay Kit

Catalog Number **MAK331**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product 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 AF HDL and LDL/VLDL Assay Kit is based on an improved PEG precipitation method in which HDL and LDL/VLDL are separated, and cholesterol concentrations are determined using a single Reaction Mix that combines cholesterol ester hydrolysis, oxidation, and color reaction in one step. The color intensity of the reaction product at 570 nm or fluorescence intensity at λ_{ex} = 530 nm/λ_{em} = 585 nm is directly proportional to total cholesterol concentration in the sample.

This kit has a linear detection range in a 96 well plate of 1–100 mg/dL cholesterol for colorimetric assays and 0.2–10 mg/dL for fluorometric assays.

Suitable for HDL and LDL/VLDL cholesterol detection in serum samples and for the evaluation of drugs on cholesterol metabolism.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96 well plates.

PBS Catalog Number MAK331A	2 × 1.5 mL
Precipitation Reagent Catalog Number MAK331B	1.5 mL
Assay Buffer Catalog Number MAK331C	20 mL
Enzyme Mix Catalog Number MAK331D	120 µL
Dye Reagent Catalog Number MAK331E	120 µL
Standard (300 mg/dL cholesterol) Catalog Number MAK331F	1 mL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Centrifuge tubes
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store all components at –20 °C upon receiving.

Procedures

Colorimetric Procedure

Note: Bring all reagents except Enzyme Mix to room temperature prior to assay.

Sample Preparation

Note: Non-hemolyzed serum samples should be used.

1. Transfer 20 μL of serum into a 1.5 mL centrifuge tube.
2. Add 20 μL of Precipitation Reagent.
3. Vortex to mix and centrifuge 5 minutes at 9,500 × *g* (e.g., 9,500 rpm in an Eppendorf® 5415C tabletop centrifuge).
4. Carefully transfer 24 μL of supernatant into a clean tube.
5. Add 96 μL of Assay Buffer. Label this tube "HDL".
6. Carefully remove all remaining supernatant from the pellet.
7. Transfer 40 μL of PBS to the pellet and mix by repeated pipetting.
8. Transfer 24 μL of mixture into another clean tube.
9. Add 96 μL of Assay Buffer. Label this tube "LDL/VLDL".
10. In a third tube, transfer 12 μL of serum sample and mix well with 108 μL of Assay Buffer. Label this tube "Total".

Standard

Transfer 5 μL of Standard (300 mg/dL cholesterol) and mix with 145 μL of Assay Buffer. Label this tube "Standard".

Reaction Mix

For each well of reaction, prepare Reaction Mix by mixing:

55 μL of Assay Buffer
1 μL of Enzyme Mix
1 μL of Dye Reagent

Assay Reaction

1. Transfer 50 μL of each of the below into separate wells of a clear flat-bottom 96 well plate. If desired, run assays in duplicate.
   Assay Buffer ("Blank")
   Standard
   "Total"
   "HDL"
   "LDL/VLDL"
2. Add 50 μL of the Reaction Mix to each standard and sample well. Tap plate to mix well.
3. Incubate 30 minutes at room temperature.
4. Measure the absorbance at 570 nm (A_{570}).
   Note: If the Sample (A_{570}) is higher than the Standard (A_{570}), dilute sample in assay buffer and repeat the assay. Multiply result by the dilution factor

Fluorometric Procedure

Note: Bring all reagents except Enzyme Mix to room temperature prior to assay.

1. Dilute the Samples and Standard prepared in Colorimetric Procedure 10-fold in Assay Buffer.
2. Transfer 50 μL of diluted standards and 50 μL of diluted samples into separate wells of a black 96 well plate.
3. Add 50 μL of Reaction Mix (see Colorimetric Procedure).
4. Tap plate to mix. Incubate 30 minutes at room temperature.
5. Read fluorescence at λ_{ex} = 530 nm/λ_{em} = 585 nm.
   Note: If the Sample fluorescence (F) is higher than the Standard fluorescence (F), dilute sample in assay buffer and repeat the assay. Multiply result by the dilution factor.

Results

Colorimetric Procedure

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

$$[\text{Total}] = \frac{A_{570\ \text{Total}} - A_{570\ \text{Blank}}}{A_{570\ \text{Standard}} - A_{570\ \text{Blank}}} \times 100\ (\text{mg/dL})$$

$$[\text{HDL}] = \frac{A_{570\ \text{HDL}} - A_{570\ \text{Blank}}}{A_{570\ \text{Standard}} - A_{570\ \text{Blank}}} \times 100\ (\text{mg/dL})$$

$$[\text{LDL/VLDL}] = \frac{A_{570\ \text{LDL/VLDL}} - A_{570\ \text{Blank}}}{A_{570\ \text{Standard}} - A_{570\ \text{Blank}}} \times 100\ (\text{mg/dL})$$

Fluorometric Procedure

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

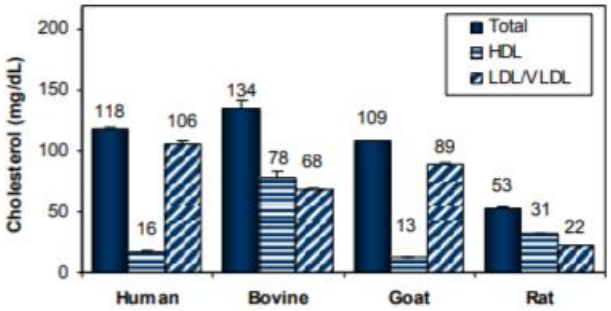
$$[\text{Total}] = \frac{F_{\text{Total}} - F_{\text{Blank}}}{F_{\text{Standard}} - F_{\text{Blank}}} \times 100\ (\text{mg/dL})$$

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

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

Figure 1.

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



References

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3. Bourdon, J.A. et al., 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 (2012).

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