

HDL and LDL/VLDL Cholesterol Quantification Kit

(Catalog #K613-100; 100 assays; Store at -20°C)

I. Introduction:

Regulation of HDL (high-density-lipoprotein)-cholesterol and LDL (low-density-lipoprotein)-cholesterol plays a central role in various disease developments. It is well known that low levels of HDL and high level of LDL are associated with an increased risk of cardiovascular events. BioVision's HDL and LDL/VLDL Cholesterol Quantification Kit provides a simple quantification method of HDL and LDL/VLDL after a convenient separation of HDL from LDL and VLDL (very low-density lipoprotein) in serum samples. In the assay, cholesterol oxidase specifically recognizes free cholesterol and produce products to react with probe to generate color($\lambda = 570$ nm) and fluorescence (Ex/Em = 538/587 nm). Cholesterol esterase hydrolyzes cholesteryl ester into free cholesterol, therefore, cholesterol ester and free cholesterol can be detected separately in the fractions by adding or not adding cholesterol esterase into the reactions.

II. Kit Contents:

Components	Volume	Cap Code	Part No.
Cholesterol Reaction Buffer	25 ml	WM	K613-100-1
2X LDL/VLDL Precipitation Buffer	10 ml	NM	K613-100-2
Cholesterol Probe (Lyophilized)	1 vial	Red	K613-100-3
Dimethylsulfoxide (DMSO; Anhydrous)	0.4 ml	Brown	K613-100-4
Enzyme Mix (Lyophilized)	1 vial	Green	K613-100-5
Cholesterol Esterase (Lyophilized)	1 vial	Blue	K613-100-6
Cholesterol Standard (5 $\mu\text{g}/\mu\text{l}$)	100 μl	Yellow	K613-100-7

III. Reagent Preparation:

Cholesterol Probe: Dissolve in 220 μl DMSO (provided) prior to use. Store at -20°C , protect from light. Use within two months.

Cholesterol Esterase: Dissolve in 220 μl Cholesterol Reaction Buffer prior to use. Aliquot and store at -20°C . Use within two months.

Enzyme Mix: Dissolve in 220 μl Cholesterol Reaction Buffer prior to use. Aliquot and store at -20°C . Use within two months.

IV. Cholesterol Assay Protocol:

1. Separation of HDL and LDL/VLDL: Mix 100 μl of 2X Precipitation Buffer with 100 μl of serum sample in micro-centrifuge tubes. After 10 min incubation at room temperature, centrifuge at 2000 x g (5000 rpm on bench-top microcentrifuge) for 10 min. Transfer the supernatant into labeled new tubes and this is the HDL fraction. The precipitates are the LDL/VLDL fraction. If you want to measure the LDL/VLDL level, the precipitates can be spun again, and the trace amount of HDL supernatant should be carefully removed, then dissolved the precipitate in 200 μl PBS. This is the LDL/VLDL fraction.

Note: A. If the supernatant is cloudy, the sample should be re-centrifuged. If the sample still remains cloudy, dilute the serum sample 1:1 with PBS, and repeat the separation procedure. Final results must be multiplied by two (2) due to the dilution with the 2X Precipitation Buffer.

B. Precipitation for longer time and precipitation/centrifugation temperature do not affect the results significantly.

2. Standard Curve and Sample Preparations: Dilute the Cholesterol Standard to 0.5 $\mu\text{g}/\mu\text{l}$ by adding 20 μl of the Cholesterol Standard to 180 μl of Cholesterol Reaction Buffer, mix well. Add 0, 4, 8, 12, 16, 20 μl into individual well in a transparent 96-well plate.

Adjust volume to total 50 μl /well with Cholesterol Reaction Buffer to generate 0, 2, 4, 6, 8, 10 μg /well of the Cholesterol Standard. (**Note:** Fluorometric assay is ~10 times more sensitive than the colorimetric assay, the cholesterol standards should be diluted 10 times further if using fluorometric assay).

For sample testing, using 2 to 20 μl of the HDL or LDL/VLDL fraction, adjust the total volume to 50 μl /well with Cholesterol Reaction Buffer. We suggest testing several different volumes of samples to ensure the readings are within the standard curve.

3. Reaction Mix Preparation: Mix enough reagents for the number of assays performed. For each assay, prepare a total 50 μl Reaction Mix containing:

- 44 μl Cholesterol Reaction Buffer
- 2 μl Cholesterol Probe**
- 2 μl Enzyme Mix
- 2 μl Cholesterol Esterase*

*Cholesterol Esterase hydrolyzes cholesteryl ester into free cholesterol. If you want to detect free cholesterol only, omit the Cholesterol Esterase in the reaction, and substitute with 2 μl of Reaction Buffer. With the addition of Cholesterol Esterase, the assay detects total cholesterol (cholesterol and cholesteryl esters). If you want to detect cholesteryl esters it self, you can subtract the value of free cholesterol from the value of total cholesterol.

Note: *For detecting either total cholesterol or free cholesterol, the Cholesterol Esterase must be added to the reaction mix for the Standard Curve reaction.

** For fluorometric assay, using 0.4 μl of Cholesterol probe for each assay can decrease fluorescence background significantly to increase detection sensitivity.

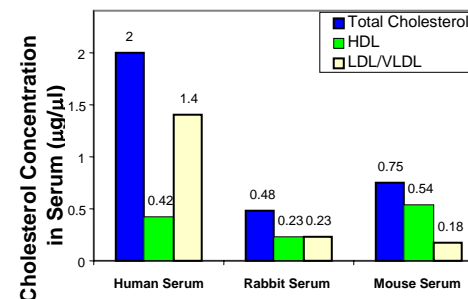
4. Add 50 μl of the Reaction Mix to each well containing the Cholesterol Standard or test samples, mix well.
5. Incubate the reaction for 60 minutes at 37°C , protect from light. Measure O.D. 570 nm (or Ex/Em 538/587 nm for fluorescence assay) in a micro-titer plate reader.
6. **Calculation:** Reagent background reading is significant, which must be subtracted from standard and sample readings. Then apply the specific sample readings to the standard curve to calculate the sample cholesterol concentrations.

$$C = A/V \mu\text{g}/\mu\text{l}; (1 \mu\text{g}/\mu\text{l} = 100 \text{mg}/\text{dL})$$

Where: A is the sample cholesterol amount from the standard curve.

V is original sample volume added in the sample well.

Free Cholesterol Molecular Weight: 386.6



Measurement of total cholesterol, HDL, LDL/VLDL from serum samples. Total Cholesterol (blue bar), HDL (green bar), and LDL/VLDL (yellow bar) cholesterol were measured following the kit protocol.