

HDL and LDL/VLDL Cholesterol Quantification Kit

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

I. Introduction:

The regulation of HDL (high-density-lipoprotein)-cholesterol and LDL (low-density-lipoprotein)-cholesterol plays a central role in various disease development. It is well known that low levels of HDL and high level of LDL are associated with an increased risk of cardiovascular events. The HDL and LDL/VLDL Cholesterol Quantification Kit provides a simple method for convenient separation of HDL and VLDL (very low-density lipoprotein) in serum sample, then HDL and LDL/VLDL cholesterol levels can easily be quantified separately, using cholesterol oxidase mediated reactions. Cholesterol esterase hydrolyzes cholesteryl ester into free cholesterol, which can then be oxidized by cholesterol oxidase to yield H₂O₂. The H₂O₂ interacts with a sensitive cholesterol probe to produce resorufin, which can be detected with a micro-plate reader at λ = 570 nm or fluorescence micro-plate reader at Ex/Em = 538/587 nm. The assay can also detect cholesterol ester and free cholesterol separately in these two 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 µg/µl)	100 µl	Yellow	K613-100-7

III. Reagent Preparation:

Cholesterol Probe: Dissolve in 220 µl DMSO (provided) prior to use. Aliquot and 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 labeled 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 clean 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. The precipitates are dissolved in 200 µl PBS and 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 µg/µl 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 total volume to 50 µl/well with Cholesterol Reaction Buffer. These 0, 2, 4, 6, 8, 10 µg/well of the Cholesterol Standard are ready to use for colorimetric assay. (Note: Since fluorescence assay is ~10 times more sensitive than colorimetric assay, the cholesterol standards should be diluted 10X more in fluorometric assay.)

For sample testing, 2 to 20 µl of the HDL or LDL/VLDL fraction should be adjusted 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 range.

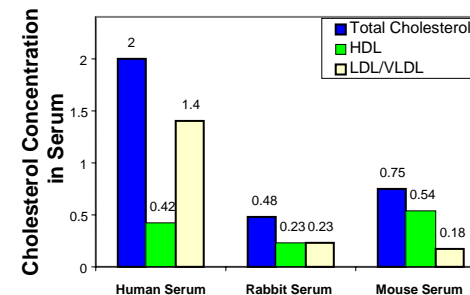
3. Reaction Mix Preparation: Mix enough reagents for the number of assays performed: For each assay, prepare a total 50 µl Reaction Mix containing:

- 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, 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.

- Add 50 µl of the Reaction Mix to each well containing the Cholesterol Standard or test samples, mix well.
- 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.
- Calculation:** Reagent background reading is significant, which must be subtracted from standard and sample readings. Then you can apply the specific sample readings to the standard curve to calculate the sample cholesterol concentrations. The concentration of HDL, LDL/VLDL cholesterol in serum should be multiplied by two (X2) due to the two times dilutions during the separation step for both HDL and LDL/VLDL fractions.



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.