

## Cholesterol/Cholesteryl Ester Quantitation Kit

(Catalog #K603-100; 100 assays; Store at -20°C)

### I. Introduction:

The Cholesterol/Cholesteryl Ester Quantitation Kit provides a simple method for sensitive quantification of cholesterol, cholesteryl Ester, or both by either colorimetric or fluorometric methods. A large portion of the cholesterol in blood is in the form of cholesteryl esters. Cholesterol esterase hydrolyzes cholesteryl ester into cholesterol. Cholesterol is then oxidized by cholesterol oxidase to yield H<sub>2</sub>O<sub>2</sub>. The produced H<sub>2</sub>O<sub>2</sub> interacts with a sensitive cholesterol probe to produce resorufin, which can be detected by spectrophotometry at  $\lambda = 570$  nm or fluorometry at Ex/Em = 535/587 nm. The assay can detect cholesterol itself (without adding cholesterol esterase) or total cholesterol (cholesterol + cholesteryl ester) by adding cholesterol esterase to the reaction, or cholesteryl ester itself by subtracting the value of cholesterol from the total value of cholesterol and cholesteryl esters.

### II. Kit Contents:

Component	Volume	Cap color	Part no.
Cholesterol Reaction Buffer	25 ml	WM	K603-100-1
Cholesterol Probe (lyophilized)	1 vial	Red	K603-100-2
Dimethylsulfoxide (DMSO; Anhydrous)	0.4 ml	Brown	K603-100-3
Enzyme Mix (lyophilized)	1 vial	Green	K603-100-4
Cholesterol Esterase (lyophilized)	1 vial	Blue	K603-100-5
Cholesterol Standard (5 µg/µl)	100 µl	Yellow	K603-100-6

### III. Storage and Handling:

Store kit at -20°C, protect from light. Allow reagents to warm to room temperature and briefly centrifuge vials before opening.

### IV. Reagent Preparation:

**Cholesterol Probe:** Dissolve in 220 µl anhydrous DMSO (provided) before use. Aliquot and store at -20°C, protect from light. Use within two months.

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

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

### V. Cholesterol Assay Protocol:

The following protocol describes assays in 100 µl per microplate well.

- Standard Curve Preparations:** For colorimetric assay, 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 each well individually. Adjust volume to 50 µl/well with Cholesterol Reaction Buffer to generate 0, 2, 4, 6, 8, 10 µg/well of the Cholesterol Standard.

For fluorometric assay, dilute the Cholesterol Standard to 50 ng/µl by adding 10 µl of the Cholesterol Standard to 990 µl of Cholesterol Reaction Buffer, mix well. Add 0, 4, 8, 12, 16, 20 µl into each well individually. Adjust volume to 50 µl/well with Cholesterol Reaction Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 µg/well of the Cholesterol Standard.

- Sample Preparations:** Prepare test samples in 50 µl/well with Cholesterol Reaction Buffer in a 96-well plate. If using serum sample, serum (0.5-2 µl/assay) can be directly diluted in the Cholesterol Reaction Buffer. If using cells or tissues or other solid samples, 10<sup>6</sup> cells or 10 mg tissue samples can be extracted by homogenization with 200 µl of chloroform:Isopropanol:Triton X-100 (7:11:0.1) in a microhomogenizer. Then spin the extract 5-10 minutes at top speed in a microcentrifuge. Collect the entire liquid phase (organic phase) without the solid phase to a new tube, air dry at 50°C to remove chloroform. Vacuum dry 30 mins to remove trace organic solvents. Dissolve the dried lipids in 200 µl of Cholesterol Reaction Buffer by vortexing extensively for 5 mins (The solution may be cloudy). The extraction procedure can be proportionally scaled up if larger amount of sample is desired. Use 1- 50 µl of the extracted sample per assay.
- Reaction Mix Preparation:** Mix enough reagent for the number of assays performed: For each well, prepare a total 50 µl Reaction Mix containing:

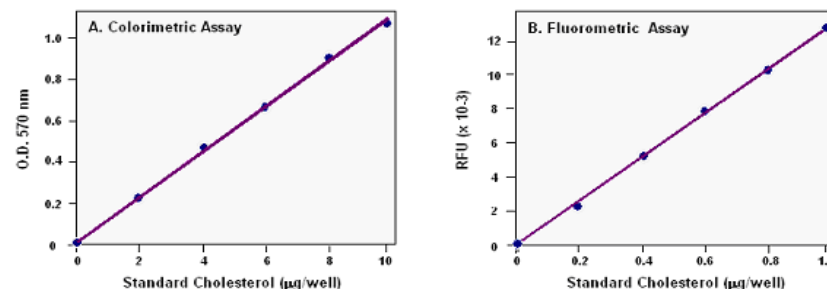
44 µl	Cholesterol Reaction Buffer
2 µl	Cholesterol Probe*
2 µl	Enzyme Mix
2 µl	Cholesterol Esterase**

\* For the fluorometric assay, use 0.4 µl of the probe for each reaction can decrease fluorescence background significantly.

\*\* Cholesterol Esterase hydrolyzes cholesteryl ester into cholesterol. If you want to detect cholesterol itself only, omit the Cholesterol Esterase. With the addition of Cholesterol Esterase, the assay detects both cholesterol and cholesteryl esters. If you want to detect Cholesteryl Esters itself, you can subtract the value of cholesterol from the total value of both cholesterol and Cholesteryl Esters.

**For standard curve, cholesterol esterase must be added to the reaction mix for detecting either total cholesterol or free cholesterol.**

- Add 50 µl of the Reaction Mix to each well containing the standard or test sample.
- Incubate the reaction for 60 minutes at 37°C, protect from light.
- Measure O.D. 570nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader.
- Correct background by subtracting the value derived from the no-cholesterol control from all samples (The background reading can be significant and must be subtracted from sample readings). Then calculate the cholesterol concentrations of the test samples based on the standard curve you generated.



**Fig. Detection of Cholesterol/Cholesteryl Ester Using Cholesterol Quantitation Kit.** Cholesterol/Cholesteryl Ester was quantified using the kit by colorimetric (A) and fluorometric (B) methods according to the kit instructions. Background from the control reaction (without cholesterol) has been subtracted from each value. **Note:** The fluorometric assay is over 10 fold more sensitive than the colorimetric assay.