For research use only

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 hydrolizes cholesteryl ester into cholesterol. Cholesterol is then oxidized by cholesterol oxidase to yield H_2O_2. The produced H_2O_2 interacts with a sensitive cholesterol probe to produce resorufin, which can be detected by spectrophotometry at λ = 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
Cholesterol Reaction Buffer	25 ml	NM
Cholesterol Probe (lyophilized)	1 vial	Red
Dimethylsulfoxide (DMSO; Dried)	0.4 ml	Brown
Enzyme Mix (lyophilized)	1 vial	Green
Cholesterol Esterase (lyophilized)	1 vial	Blue
Cholesterol Standard (5 μg/μl)	100 μl	Yellow

III. Storage and Handling:

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

IV. Reagent Preparation:

Cholesterol Probe: Dissolve in 220 μl 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.

2. **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, 10^6 cells or 10 mg tissue can be extracted with 200 μl of chloroform-methanol (2:1) or 200 μl of hexane-isopropanol (3:2). Spin down to collect supernatant, vacuum dry, then dissolve the dried lipids in 200 μl of 2-propanol containing 10% Triton X-100 as assay samples.

3. **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*

   *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.

4. Add 50 μl of the Reaction Mix to each well containing the Cholesterol standard or test sample.

5. Incubate the reaction for 60 minutes at 37°C, protect from light.

6. Measure O.D. 570nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader.

7. 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.

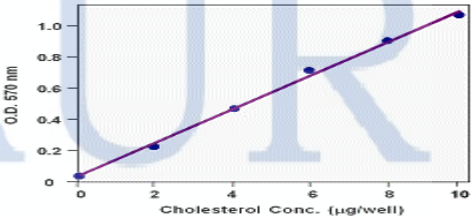

Fig. 1. Detection of Cholesterol/Cholesteryl Ester Using Cholesterol Quantitation Kit. Cholesterol/Cholesteryl Ester was quantified using the Cholesterol probe according to the kit instructions. Background from the control reaction (without cholesterol added) has been subtracted from each value.

Note: Fluorometric assay is 4-10 folds more sensitive than colorimetric assay, can detect 0.02-1 μg/well.