Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay Kit (BN00828)

(Catalog # BN00828; 100 assays; Store at -20°C)

I. Introduction:

The Cholesterol/Cholesteryl Ester Quantitation Kit provides a simple method for sensitive quantification of free cholesterol, cholesteryl esters, or both by colorimetric or fluorometric methods. Majority of the cholesterol in blood is in the form of cholesteryl esters which can be hydrolyzed to cholesterol by cholesterol esterase. Cholesterol is then oxidized by cholesterol oxidase to yield H_2O_2 which reacts with a sensitive cholesterol probe to produce color (OD 570 nm) and fluorescence (Ex/Em = 535/587 nm). The assay detects total cholesterol (cholesterol and cholesteryl esters) in the presence of cholesterol esterase or free cholesterol in the absence of cholesterol esterase in the reaction. Cholesteryl ester can be determined by subtracting the value of free cholesterol from the total (cholesterol plus cholesteryl esters).

II. Application:

- Measurement of cholesterol in various tissues/cells
- Analysis of lipid metabolism in various cells

III. Sample Type:

- Animal tissues
- Cell culture: Adherent or suspension cells
- Serum

IV. Kit Contents:

Components	BN00828	Cap Code	Part Number
Cholesterol Assay Buffer	25 ml	WM	BN00828-1
Cholesterol Probe (in DMSO, anhydrous)	200 µl	Red	BN00828-2A
Enzyme Mix (lyophilized)	1 vial	Green	BN00828-4
Cholesterol Esterase (lyophilized)	1 vial	Blue	BN00828-5
Cholesterol Standard (2 µg/µl)	100 µl	Yellow	BN00828-6

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom
- Multi-well spectrophotometer

VI. Storage and Handling:

Store kit at –20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the experiment. Keep enzymes and cholesterol standard on ice while using.

VII. Reagent Preparation and Storage Conditions:

- **Cholesterol Assay Buffer:** Warm to room temperature before use. Store at 4° C or -20° C.
- **Cholesterol Probe:** Ready to use as supplied. Warm to room temperature to thaw the DMSO solution before use. Store at –20°C, protect from light. Use within two months.
- **Enzyme Mix:** Dissolve in 220 µl Cholesterol Assay Buffer before use. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.
- **Cholesterol Esterase**: Dissolve in 220 µl Cholesterol Assay Buffer before use. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.
- **Cholesterol Standard:** Keep on ice while in use.

VIII. Cholesterol Assay Protocol:

1. **Sample Preparation:** Serum samples (0.5-5 µl/assay) should be diluted 10-fold in the Cholesterol Assay Buffer. For cells or tissue samples, 10^6 cells or 10 mg tissue can be extracted with 200 µl of chloroform : Isopropanol : NP-40 (7:11:0.1) in a micro-homogenizer. Spin the extract 5-10 min. at 15,000 x g in a centrifuge. Transfer all of the liquid (organic phase) avoiding the pellet, to a new tube, air dry at 50°C to remove chloroform. Put the samples under vacuum for 30 min. to remove trace organic solvent. Dissolve dried lipids with 200 µl of Cholesterol Assay Buffer by sonicating or vortexing until homogeneous (it is OK if the solution becomes cloudy). The extraction procedure can be scaled up if larger amounts of sample are desired. Use 1- 50 µl of extracted sample per assay. Then adjust the volume to 50 µl/well with Cholesterol Assay Buffer.

   Notes:

   a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.

   b. For samples having background, prepare parallel well(s) containing same amount of sample as in the test well.

   c. Endogenous compounds may interfere with the reaction. To ensure accurate determination of Cholesterol in the test samples, we recommend spiking samples with a known amount of Standard (2 µg).

2. **Standard Curve Preparation:** For the colorimetric assay, dilute the Cholesterol Standard to 0.25 µg/µl by adding 20 µl of the Cholesterol Standard to 140 µl of Cholesterol Assay Buffer, mix well. Add 0, 4, 8, 12, 16, 20 µl into a series of wells. Adjust volume to 50 µl/well with Cholesterol Assay Buffer to generate 0, 1, 2, 3, 4, 5 µg/well of the Cholesterol Standard.

For the fluorometric assay, dilute the Cholesterol Standard to 25 ng/µl by adding 10 µl of the Cholesterol Standard to 790 µl of Cholesterol Assay Buffer, mix well. Follow the same protocol above to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 µg/well of the Cholesterol Standard.

3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 100 µl Reaction Mix containing:

	Reaction Mix	*Background Control Mix
Cholesterol Assay Buffer	44 µl	46 µl
Cholesterol Probe[1]	2 µl	2 µl
Cholesterol Enzyme Mix	2 µl	---
Cholesterol Esterase[2,3]	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing standard or test samples.

* For samples having background, add 50 µl of Background Control Mix to sample background control well(s)

Notes:

1. For the fluorometric assay, use 0.4 µl of the probe for each reaction to decrease fluorescence background. The fluorometric assay is over 10 fold more sensitive than the colorimetric assay.
2. Cholesterol Esterase hydrolyzes cholesteryl ester to cholesterol. If you want to detect free cholesterol only, omit the Cholesterol Esterase in the reaction. In the presence of Cholesterol Esterase, the assay detects both free cholesterol and cholesteryl esters. If you want to determine Cholesteryl Ester only, subtract the value of free cholesterol from the value of total cholesterol (Cholesterol and Cholesteryl Ester).
3. The Cholesterol Standard contains a mixture of free cholesterol and cholesterol esters in a similar ratio of serum. Cholesterol Esterase must be added to the standard curve reaction to convert all cholesterol.

4. **Measurement:** Incubate the reaction for 60 min. at 37°C, protect from light. Measure absorbance at 570 nm for the colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader.

5. **Calculation:** Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample readings. Plot the Cholesterol Standard Curve. For unspiked samples, apply the corrected absorbance or fluorescence to the Cholesterol Standard Curve to get B µg of Cholesterol in the sample well.

Sample Cholesterol concentration (C) = B/V X D (µg/µl)

Where: **B** is the amount of Cholesterol in the sample well (µg)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Cholesterol amount in sample well (B) = $\left(\frac{(\mathrm{OD}_{\text{sample (corrected)}})}{(\mathrm{OD}_{\text{sample + Chol Std(corrected)}})-(\mathrm{OD}_{\text{sample(corrected)}})}\right) * Chol\ Spike\ (\mu g)$

Cholesterol Molecular weight: 386.15. 1 µg/µl= 100 mg/dl.

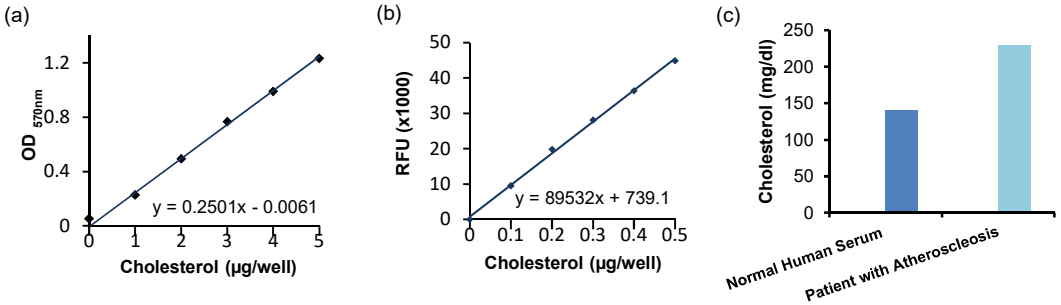

Figure: Cholesterol Standard Curve, colorimetric (a) and fluorometric (b). (c) Quantification of Cholesterol/Cholesterol Ester from normal human serum (1 µl) and serum from patient with atherosclerosis (1 µl). Assay was performed following the kit protocol.

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