Product Information

Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2090-100 100 assays	Cap Color	Part Number
Cholesterol Assay Buffer	25 ml	WM	K2090-C-1
Cholesterol Probe (in DMSO, anhydrous)	200 µl	Red	K2090-C-2
Enzyme Mix (lyophilized)	1 vial	Green	K2090-C-3
Cholesterol Esterase (lyophilized)	1 vial	Blue	K2090-C-4
Cholesterol Standard (2 µg/µl)	100 µl	Yellow	B1702

II. Introduction:

Cholesterol is a sterol molecule and is an important structural component of all animal cell membranes to maintain both membrane structural fluidity and integrity. Cholesterol also acts as a precursor for the biosynthesis of vitamin D, steroid hormones and bile acids. Cholesteryl ester is an ester of cholesterol and can be hydrolyzed by pancreatic enzymes and cholesterol esterase to produce cholesterol and free fatty acids.

The Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay Kit provides a sensitive, simple and convenient way for detection of free cholesterol, cholesteryl esters, or both in various biological fluids based on colorimetric and fluorometric method. Majority of the cholesterol in blood exists in the form of cholesteryl esters which can be hydrolyzed to free cholesterol and fatty acids by cholesterol esterase. Cholesterol is then oxidized by cholesterol oxidase to generate H_2O_2 which reacts with a sensitive cholesterol probe to yield color (λmax = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The assay can detect free cholesterol in the absence of cholesterol esterase or total cholesterol (cholesterol and cholesteryl esters) in the presence of cholesterol esterase in the reaction. Cholesteryl ester is determined by subtracting the value of free cholesterol from the total cholesterol.

III. Application:

Measurement of cholesterol in various tissues/cells.

Analysis of lipid metabolism in various cells.

IV. Sample Type:

Animal tissues.

Cell culture: Adherent or suspension cells Serum.

V. User Supplied Reagents and Equipment:

96-well plate with flat bottom.

Multi-well spectrophotometer.

VI. Storage and Handling:

Store kit at –20℃, 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℃ or -20℃.

Cholesterol Probe: Ready to use as supplied. Warm to room temperature to thaw the DMSO solution before use. Store at –20℃, protect from light. Use within two months.

Enzyme Mix: Dissolve in 220 µl Cholesterol Assay Buffer before use. Aliquot and store at –20℃. 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℃. 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 microhomogenizer. 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℃ 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 Probe1	2 µl	2 µl
Cholesterol Enzyme Mix	2 µl	---
Cholesterol Esterase2,3	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:

a. For the fluorometric assay, use 0.4 µl of the probe for each reaction to decrease fluorescenc background. The fluorometric assay is over 10 fold more sensitive than the colorimetric assay.

b. 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).

c. 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℃, 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) = ($OD_{sample\ (corrected)}$/ ($OD_{sample + Chol\ Std(corrected)}$× $OD_{sample\ (corrected)}$))* Chol Spike (µg)

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

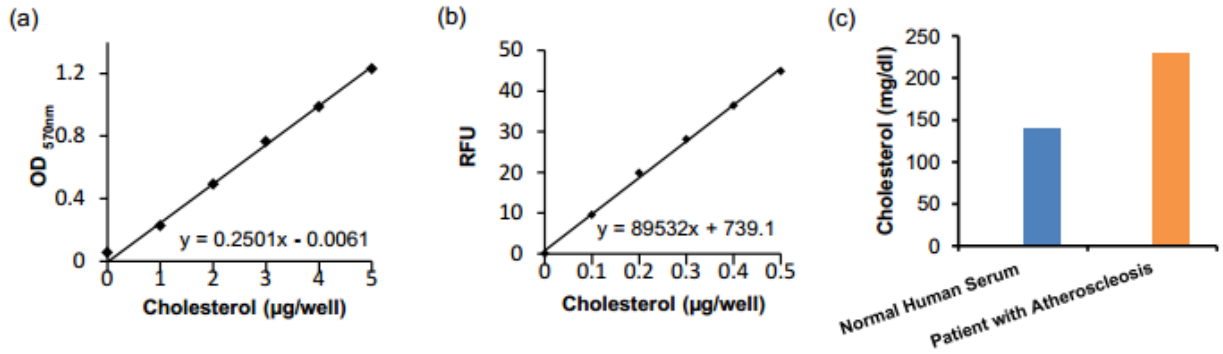

Figure: Cholesterol Standard Curve, colorimetric (a) and fluorometric (b). (c) Quantification of Cholesterol/Cholesterol Ester from normal human serum (1 µl) and serum from patient with atherosclerosis (1 µl). Assay was performed following the kit protocol.

Frequently Asked Questions:

1. The enzyme does not fully dissolve in 220 µl of Assay Buffer. Will this affect the results?

The enzyme solution is likely super-saturated and hence some material is insoluble. We have tested the assay with 100% solubilized enzyme and 50% solubilized enzyme and the results were identical. The enzyme solution can be warmed up briefly for 1 - 2 minutes at 37℃ to help solubilize more enzyme.

2. The probe changes to red color after dissolving. Does this affect the assay?

The probe changing to red color after dissolving in DMSO should not affect the quality of the assay. The probe will always be pink to red and the extent of redness depends on the level of oxidation. However, a pinkish/mild red color should not affect the assay significantly.

3. To measure free cholesterol and total cholesterol, are two separate sets of standard curves needed?

No. Only one standard curve after adding the cholesterol esterase should be produced.

4. In serum both esterified and non-esterified cholesterol is detectable. From tissue homogenates only the non-esterified version is detected. Why?

When tissues are homogenized, cellular enzymes including Esterases are released. These enzymes hydrolyze the esterified cholesterol to non-esterified cholesterol.

5. Can EDTA be used for blood collection for this assay?

Yes, EDTA will be fine.

6. Can this kit measure free or protein-bound cholesterol?

This kit can measure both free and apolipoprotein-bound forms of cholesterol.

7. What is the detailed principle of measurement?

Cholesterol esterase hydrolyzes cholesterol ester into free cholesterol. Then cholesterol oxidase in the enzyme mix oxidizes the free cholesterol to generate H_2O_2 .H_2O_2 then interacts with the cholesterol probe to generate color/fluorescence.

8. Can this kit be used with tissue samples, e.g. liver?

Yes, tissue samples can be used with this kit. It is critical to homogenize the tissue in chloroform:Isopropanol: NP-40 for effective extraction of lipids from the sample. Please follow the instructions on the Datasheet for further details.

9. Can the Absorbance for this kit be read at 620 nm instead of 570 nm?

All spectrophotometers have a window within which they are equally accurate. Typically this window is +/-20 to +/-40 nm. 620 nm seems a bit far-stretched for 570 nm recommended wavelength for this assay but I would suggest checking the instrument specifications.

10. Can frozen tissue be used with this kit?

Although not ideal, flash-frozen tissue or cells stored at -80℃ can be used. It is important to remember that results may vary between fresh and frozen samples.

11. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

12. Can alternate buffers for sample preparation (cell lysis, sample dilutions etc) be used?

Our assay buffers are optimized for the reactions in this assay. They not only contain some detergents for efficient lysis of your cells, but also contain some proprietary components required for the detection reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

13. Is it essential to make a standard curve for every expt, or is one curve/kit enough?

Yes, we strongly recommend you measure the standards every time you do an experiment. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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