Product Information

Total Cholesterol and Cholesteryl Ester Colorimetric Assay Kit II

I. Kit Contents:

Components	K2128-100 100 assays	Cap Color	Part Number
Cholesterol Assay Buffer	25 ml	WM	K2128-C-1
Substrate Mix (lyophilized)	1 vial	Red	K2128-C-2
Enzyme Mix (lyophilized)	1 vial	Green	K2128-C-3
Esterase (lyophilized)	1 vial	Blue	K2128-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 Assay Kit II provides a sensitive, simple and convenient way for detection of free cholesterol, cholesteryl esters, or both in various biological fluids based on colorimetric 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. In the assay, cholesterol is oxidized by cholesterol dehydrogenase to produce NADH which reacts with a sensitive probe to yield color (λmax = 450 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. The probe is more sensitive, stable and specific. The assay can tolerate interferences from various samples significantly.

III. Storage and Handling:

Store kit at -20℃, protect from light. Allow Assay Buffer to warm to room temperature before use. Keep enzymes and cholesterol standard on ice while using. Read the entire protocol before perform the assay.

IV. Reagent Preparation:

Substrate Mix: Reconstitute with 220 µl of Assay Buffer and mix thoroughly. The solution is stable for 2 months at 4℃.

Enzyme Mix: Dissolve in 220 µl Cholesterol Assay Buffer before use. Aliquot and store at -20℃. Use within two months.

Esterase: Dissolve in 220 µl Cholesterol Assay Buffer before use. Aliquot and store at -20℃. Use within two months.

V. Cholesterol Assay Protocol:

1. Standard Curve Preparations: 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.

2. Sample Preparation: Serum should be diluted 10X with Cholesterol Assay Buffer, then use 2 - 20 µl for each testing. For cells or tissue samples, extract 10^6 cells or 10 mg tissue with 200 µl.

CHCl3: IPA:NP-40 (7:11:0.1) in a microhomogenizer. Centrifuge the extract for 5 min at 15,000xg. Transfer the liquid phase to a new tube and air dry at 50℃ then place samples under vacuum for 30 min to remove any remaining solvent. Dissolve dried lipids with 200 µl of Cholesterol Assay Buffer by sonicating or vortexing until homogeneous (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 extract per assay. Adjust volume to 50 µl/well with Cholesterol Assay.

Buffer. For unknown samples, we suggest testing different amounts of sample to ensure that readings are within the limits of the standard curve.

3. Ester Hydrolysis: Add 2 µl Esterase to each standard and samples for which the total cholesterol value is desired. (See Notes a and b in step 4). Incubate 30 min at 37℃.

4. Reaction Mix Preparation: Mix enough reagent for the number of assays performed:

For each well, prepare a total 48 µl Reaction Mix containing:

Cholesterol Assay Buffer	44 µl
Substrate Mix	2 µl
Cholesterol Enzyme Mix	2 µl

Mix. Add 48 µl of the Reaction Mix to each well containing standard or samples.

Notes:

a) Cholesterol Esterase hydrolyzes cholesteryl ester to cholesterol. If you want to detect free cholesterol only, omit the Cholesterol Esterase in the reaction (step 3) & adjust the volume of reaction mix to 50 µl by adding 46 µl Cholesterol Assay Buffer. 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).

b) The Cholesterol Standard contains a mixture of free cholesterol and cholesterol esters in a similar ration of serum. Cholesterol Esterase must be added to the standard reaction to convert all cholesterol in the standard.

5. Incubate the reaction for 30 min at 37℃, protect from light.

6. Measure absorbance at 450 nm in a microplate reader.

7. Calculations: Subtract the 0 standard background reading from all readings. Plot the standard curve. Apply sample readings to the standard curve. Cholesterol concentration in samples can then be calculated:

C = A/V X D (µg/µl)

Where: A = amount of cholesterol determined from Standard Curve (in µg).

V = volume of sample added into the reaction well (in µl).

D = Sample dilution factor.

Cholesterol molecular weight: 386.65. 1µg/µl = 100 mg/dL.

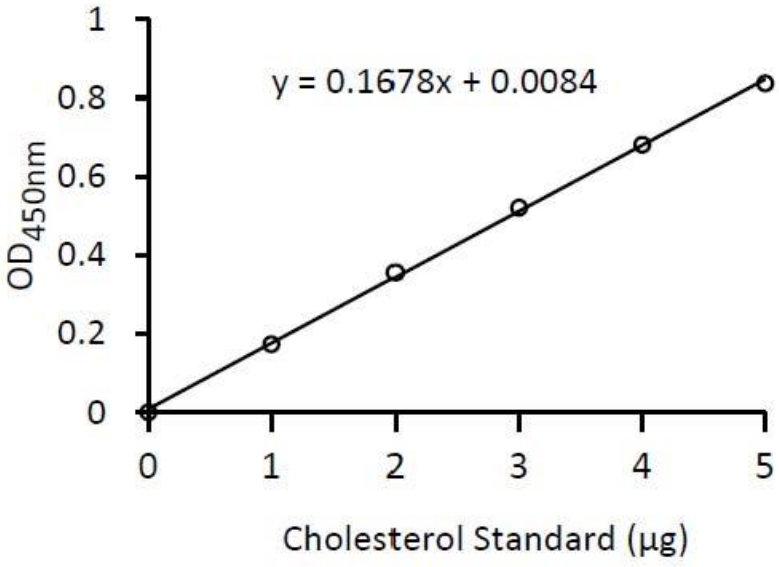

Figure: Cholesterol Standard Curve: Cholesterol/Cholesteryl Ester was quantified according to the kit instructions. Background from the 0 standard reading (without cholesterol) has been subtracted from all readings.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and

		temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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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.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com