Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay Kit

For the measurement of cholesterol in various biological samples (tissues/cells) and analysis of lipid metabolism in various cells.

For research use only - not intended for diagnostic use.

Storage and Stability

Entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer II/Cholesterol Assay Buffer	25 mL	-20°C
OxiRed Probe/Cholesterol Probe (in DMSO, anhydrous)	0.2 mL	-20°C
Enzyme Mix I/Enzyme Mix (lyophilized)	1 vial	-20°C
Cholesterol Esterase (lyophilized)	1 vial	-20°C
Cholesterol Standard (2 μg/μl)	100 μl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability

Reagent Preparation Before using the kit, spin the tubes prior to opening. Keep enzymes and cholesterol standard on ice while using.

Assay Buffer II/Cholesterol Assay Buffer: Warm to room temperature prior to use. Store at -20°C or 4°C.

OxiRed Probe/Cholesterol Probe: Ready to use as supplied. Warm to room temperature to thaw the OxiRed Probe/probe solution prior to use. Store at -20°C, protect from light. Use within two months.

Enzyme Mix I/Enzyme Mix: Dissolve in 220 µl Assay Buffer II/Cholesterol Assay Buffer. Aliquot & store at -20°C. Keep on ice while in use. Use within two months.

Cholesterol Esterase: Dissolve in 220 µl Cholesterol Assay Buffer. Aliquot & store at -20°C. Keep on ice while in use. Use within two months.

Cholesterol Standard: Keep on ice while in use.

Assay Protocol

Sample Preparation

Serum samples: (0.5-10 µl/assay) should be diluted 10-fold in the Assay Buffer II/Cholesterol Assay Buffer. Use 1-10 µl of diluted sample per well. Adjust the volume to 13.0 µl with Assay Buffer II/Cholesterol assay buffer.

Notes:

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

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

− Instrument reader settings need to be adjusted according to the chosen 384-well plate.

Standard Curve Preparation

1. Dilute the Cholesterol Standard to 125 ng/µl by adding 10 µl of the Cholesterol Standard to 150 µl of Assay Buffer II/Cholesterol Assay Buffer, mix well.

2. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 384-well plate to generate 0, 250, 500, 750, 1000, and 1250 ng/well of Cholesterol Standard. Adjust volume to 13.0 µl/well with Assay Buffer II/Cholesterol Assay Buffer.

Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 12.0 µl Mix containing:

Item	Reaction Mix	Free Cholesterol**	Background control mix*
Assay Buffer II/Cholesterol Assay Buffer	10.5 µl	11 µl	11 µl
OxiRed Probe/Cholesterol Probe	0.5 µl	0.5 µl	0.5 µl
Enzyme Mix I/Enzyme Mix	0.5 µl	0.5 µl	---
Cholesterol Esterase	0.5 µl	----	0.5 µl

Mix and add 12.0 µl of the Reaction Mix to each well containing the Standard, or test samples.

Mix well.

* For samples having background, add 12.0 µl of the background control mix to the sample background control well(s)

Notes:**

− Cholesterol esterase hydrolyses cholesteryl ester to cholesterol. Cholesterol esterase is used to detect both free cholesterol and cholesteryl esters. For detecting free cholesterol only: prepare a reagent mix containing the Enzyme Mix I/enzyme mix only, as indicated above. For detecting Cholesterol esters only: subtract the value of free

cholesterol from the value of the total cholesterol (cholesterol and cholesteryl esters).

− The Cholesterol Standard contains a mixture of free cholesterol and cholesteryl esters in a ratio similar to that of serum. Cholesterol Esterase must be added to the standard curve reaction to convert all to cholesterol.

Measurement: Incubate the reaction for 60 min. at 37°C, protect from light. Measure absorbance (OD: 570 nm) in a microplate reader.

Calculations:

1. Subtract 0 Standard reading from all readings which will be the corrected absorbance readings.

2. If the sample background control reading is significant then subtract the sample background control reading from the sample reading.

3. Plot the Cholesterol Standard Curve (OD: 570 nm vs ng Standard).

4. Apply the corrected absorbance of the sample to the Cholesterol Standard Curve to get B ng of Cholesterol in the sample well.

Sample Cholesterol concentration (C) = B/V x D (ng/µl)

Where:

B = is the amount of Cholesterol in the sample well from Standard Curve

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

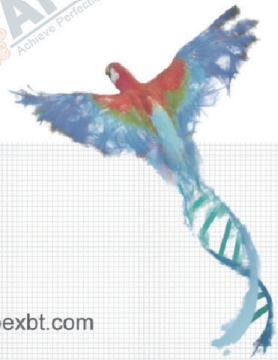
D = sample dilution Factor

Cholesterol molecular weight: 386.15 g/mol. 1000 ng/ µl = 1 µg/ µl = 100 mg/dl**10 mM ≡** 2.56 mg/ml

Technical Support

For research use only! Not to be used in humans.

For more details, please visit http://www.apexbt.com/or contact our technical team.



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