Total Cholesterol

Microplate Assay Kit

User Manual

Catalog # FTA0114

(Version 1.2C)

Detection and Quantification of Total Cholesterol (TC) Content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Cholesterol is a sterol and lipid present in the cell membranes, and is transported in the bloodstream of all animals. It is used to form cell membranes and hormones, and plays important roles in cell signaling processes. Elevated levels (hypercholesterolemia) have been associated with cardiovascular diseases such as atherosclerosis; whereas, low levels (hypocholesterolemia) may be linked to depression, cancer and cerebral hemorrhage.

In this kit, the cholesterol concentration is determined by a coupled enzyme assay. The products can be measured at a colorimetric readout at 550 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Diluent	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
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Note:

Enzyme: add 9 ml Diluent to dissolve before use.

Dye Reagent: add 10 ml Diluent to dissolve before use.

Standard: add 1 ml Assay Buffer to dissolve before use; then add 0.5 ml into 0.5 ml Assay Buffer, mix, the concentration will be 5 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. SAMPLE PREPARATION

1. For serum, plasma and other biological samples

Detect directly. Dilute samples 10-fold (e.g. 10 µl sample with 90 µl Assay Buffer).

2. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Assay Buffer	--	--	10 µl
Enzyme	90 µl	90 µl	--
Diluent	--	--	90 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, 37 °C wait for 10 minutes, measured at 550 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

1. According to the liquid sample

$TC\,(mmol/L) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$

$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the weight of sample

$TC\,(mmol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$

$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the concentration of cell or bacteria

$TC\,(mmol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$

$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$C_{Standard}$: the concentration of Standard, 5 mmol/L;

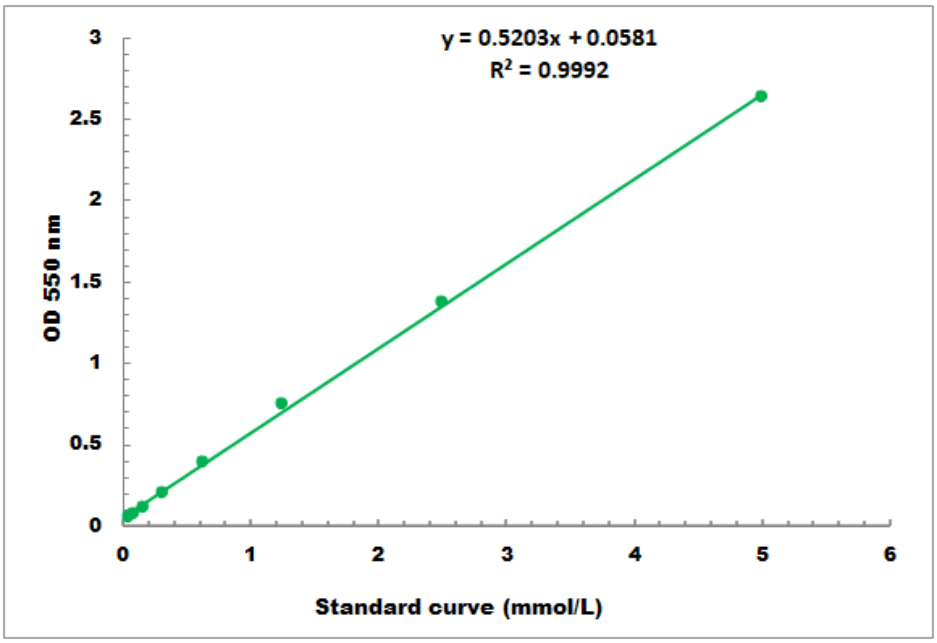
$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES