Total Cholesterol

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8014

(Version 1.1A)

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.

Total Cholesterol Fluorometric Microplate Assay Kit is a sensitive assay for determining Total Cholesterol in various samples. In the assay, the cholesterol can be hydrolyzed to cholesterol by cholesterol esterase. Cholesterol is then oxidized by cholesterol oxidase to yield H2O2, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	--
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	--
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Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within one month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within one month.

Standard: add 1 ml Assay Buffer to dissolve before use; then add 20 µl into 980 µl Assay Buffer, mix, the concentration will be 0.2 mmol/L. Store at -20 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
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%, sonicate 3s, interval 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

Warm the solution to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	170 µl	170 µl	170 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Probe	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 10 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.			

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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the liquid sample

$TC\,(\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$

$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the weight of sample

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

$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the concentration of cell or bacteria

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

$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

4. According to the protein concentration of sample

$TC\,(\mu mol/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$

$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

$C_{Standard}$: the concentration of Standard, 0.2 mmol/L = 0.2 μmol/ml

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

W: the weight of sample, g

N: the quantity of cell or bacteria, $N \times 10^4$

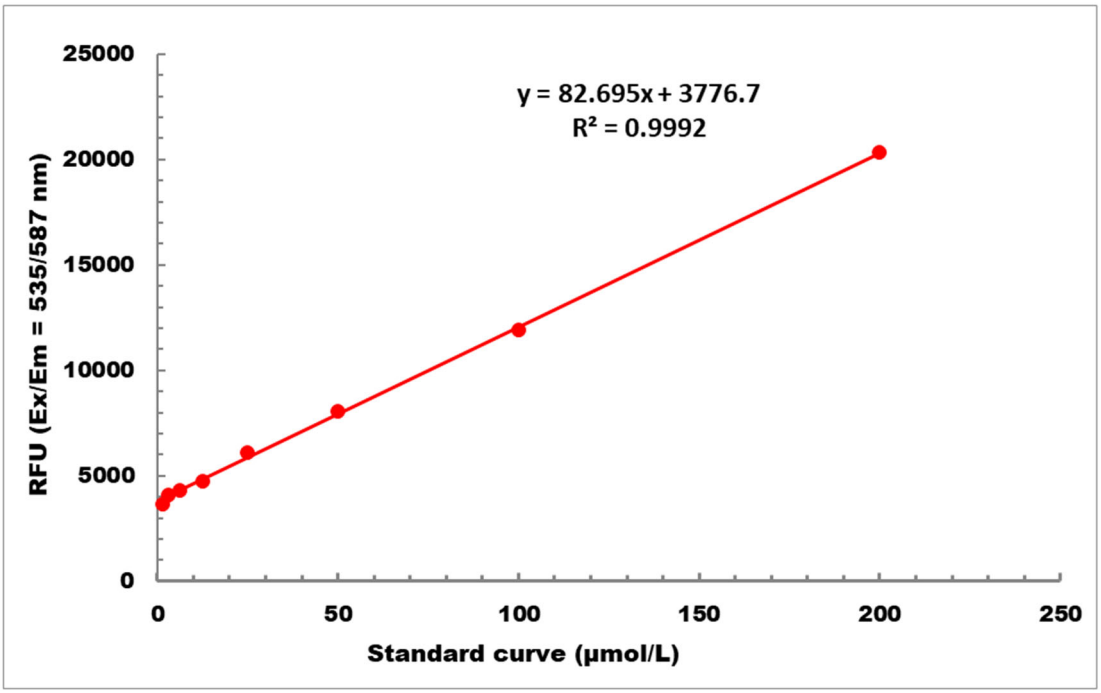
$V_{Standard}$: the volume of standard, 0.01 ml

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: 2 µmol/L - 200 µmol/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