Total Cholesterol Fluorometric Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6903

Detection and Quantification of Total Cholesterol (TC)Content inSerum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

1. 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 b een associated with cardiovascular diseases such as atherosclerosis; whereas, low levels (hypocholesterolemia) may belinked 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 canbe hydrolyzed to cholesterol by cholesterol esterase.Cholesterol is then oxidized by cholesterol oxidase to yield H_2O_2 , which can be detected fluorometrically (Ex/Em 535/587).

2. 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, keep in dark
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 mlx 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 strips	--
Technical Manual	1 Manual	-

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL Assay Buffer to generate 10 mmol/ L of standardstock solution,store at -20°C for 4-5 month safter reconstitution. Then dilute to 200 μmol/ L standard top solution by adding 20 μl stock solution into 980μl Assay Buffer.Perform 2 -fold serial dilutions of the top standardsolution using Assay Buffer to make the standard curve.The concentration of standard curve could be 200/100/50/25/12.5/6.25/3.12μmol/ L.

Enzyme: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use. Aliquot & store at -20°C.Use within 1 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 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. 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 8000 g 4°C for 10minutes , 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 8000 g 4°C for 10minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

6. Assay Procedure

Warm thesolution 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

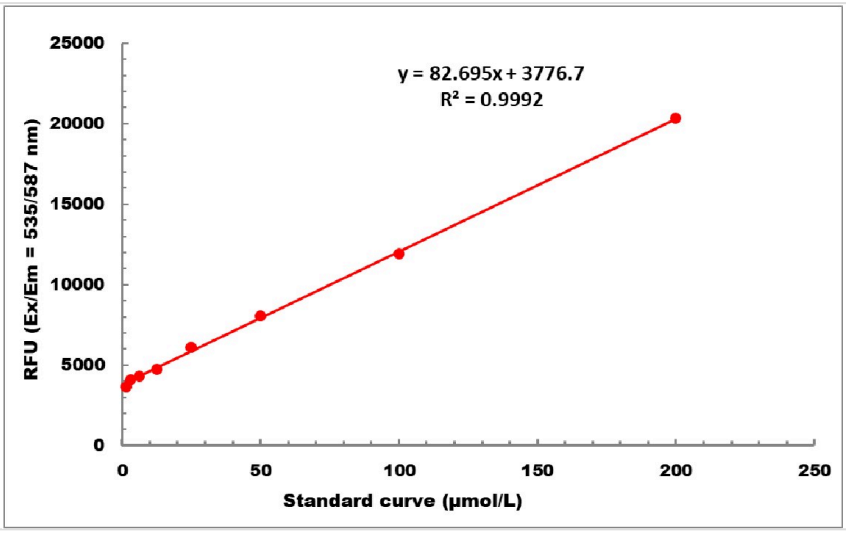
2.4According to the volume of sample

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

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, μmol/L
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl
V_{Assay}	the volume of Assay Buffer, μl
$C_{Protein}$	the sample protein concentration, mg/ml
W	the weight of sample, g
N	the quantity of cell or bacteria, 10^4

8. Typical Data

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



Detection Range : 2μmol /L-200μmol /L