Total Cholesterol

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1116

(Version 1.5C)

Detection and Quantification ofTotal Cholesterol (TC)ContentinSerum, 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 belinked to depression, cancer and cerebral hemorrhage.

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

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Diluent	20 mlx 1	4 °C
Enzyme	Powderx 1	-20 °C, keep in dark
Dye Reagent	Powderx 1	4 °C, keep in dark
Standard	Powderx 1	4 °C
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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. REAGENT PREPARATION

Enzyme: Add 9 ml Diluent to dissolve before use. Store at 4 °C for 1 dayor -20 °C for 4months.

Dye Reagent: Add 10 ml Diluent to dissolve before use. Store at 4 °C for 2-3weeksor -20 °C for 2months.

Standard:Briefly centrifuge prior to opening. Add 1 mlAssay Buffer to dissolve before use; then add 0.5 ml into 0.5 mlAssay Buffer, mix, theconcentration will be 5 mmol/L.Perform 2-fold serial dilutions of the top standardsolution using Assay Bufferto make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.3125/0.156 mmol/L.

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

V. SAMPLE PREPARATION

1.For cell samples

Collect cells into centrifuge tube, discard the supernatant after centrifugation, add 0.5 mldistilled water for 5 ×10^6 cells, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times). Mix the sample homogenate with 0.5 ml Assay Buffer and vortex thoroughly. Centrifuged at 10,000g 4°C for 10 minutes, then carefully transfer the upper heptane phase into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 0.5 ml distilled wateron iceadequately.Mix the sample homogenate with 0.5 mlAssay Buffer and vortex thoroughly. Centrifuged at 10,000g 4°C for 10 minutes, then carefully transfer the upper heptane phase into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directlyordilute samples with Assay Buffer,centrifuged at 10,000g 4°C for 10 minutes, then transfer the upper heptane phase into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

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

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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

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

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

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

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

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

Slope: the absorbance slope of standard curve

n: the dilution factor

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

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume Assay Buffer in sample preparation, μl

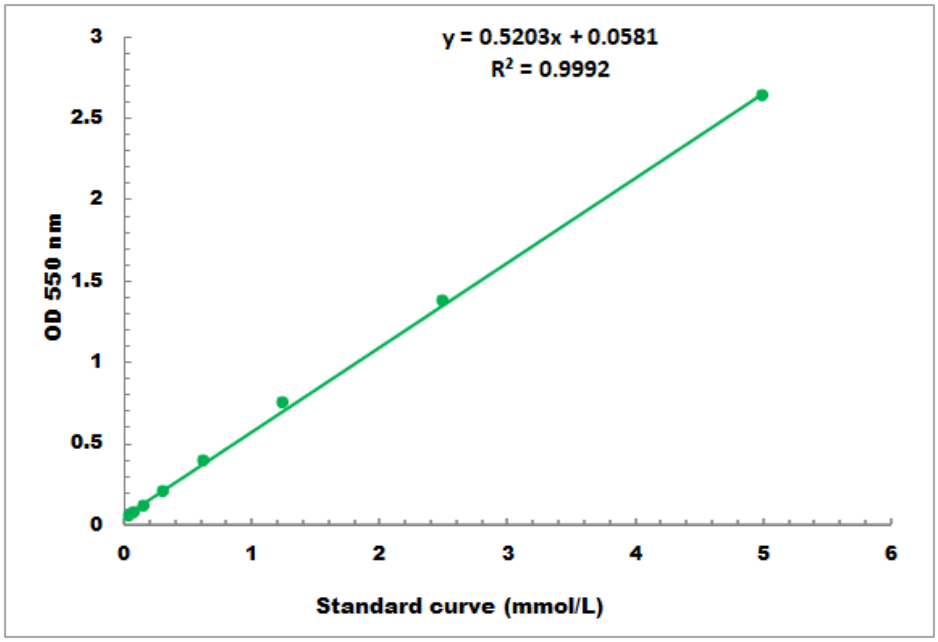
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

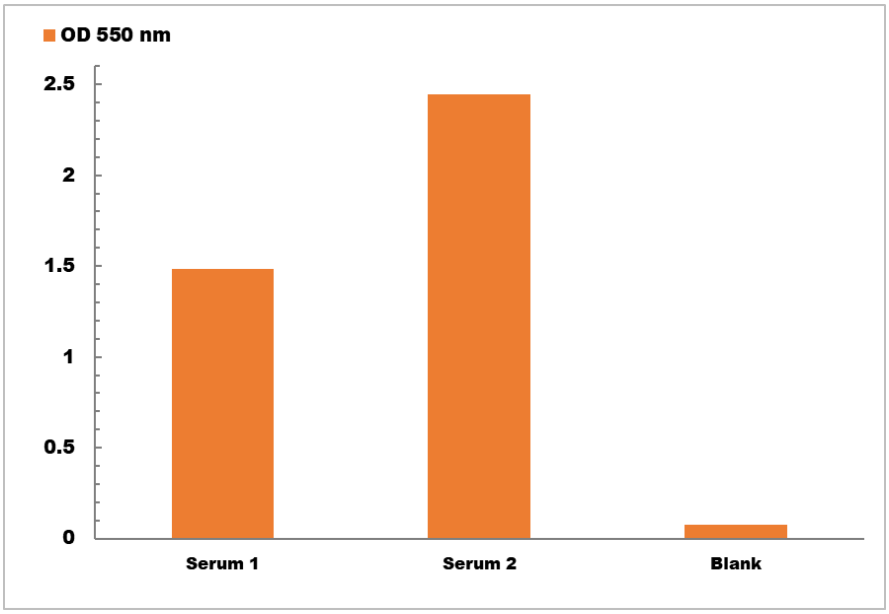
N: the quantity of cell, 10^4

VIII. 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



Determination of total cholesterol in serum