

Total Cholesterol (TC) Content Assay Kit

Product code: 112612

Method: Spectrophotometric method

Product Introduction

Total cholesterol (TC) includes free cholesterol and cholesteryl esters. TC refers to the total cholesterol contained in all lipoproteins in the tissue.

In this assay, cholesteryl esters are hydrolyzed by esterase to produce free cholesterol (FC) and free fatty acids (FFA). The FC is then oxidized by cholesterol oxidase to generate Δ^4 -cholestenone and H_2O_2 . Finally, peroxidase catalyzes H_2O_2 to oxidize 4-aminoantipyrine and phenol, producing a red quinone compound. This compound has a characteristic absorption peak at 500 nm, and the color intensity is directly proportional to the TC content.

Package Contents

Pack Size	Code	Item	Quantity	Notes
50T	112612.1	Reagent I	User prepared	Isopropanol
50T	112612.2	Reagent II	1 bottle	
50T	112612.3	Reagent III	1 bottle	
50T	112612.4	Reagent IV	1 bottle	
50T	112612.5	TC Standard	1 bottle	
50T	112612.m	Manual	1 copy	

Quality Standards and Safety Instructions

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Reagent II	-	-
Reagent III	-	-
Reagent IV	-	-
TC Standard	-	-

Transportation and Storage Conditions

Item	Condition
Transport	Transport with ice packs.
Storage	Store at 2-8 °C, protected from light.
Shelf Life	180 days.

Instructions for Use

1. Total Cholesterol Extraction

- 1. Tissue samples:** Extract TC from tissue according to a tissue mass (g) to Reagent I volume (mL) ratio of 1:5-10. It is recommended to weigh about 0.1 g tissue and add 1 mL Reagent I. Homogenize in an ice bath, centrifuge at 8000 g and 4 °C for 10 min, then collect the supernatant as the test sample.

2. **Cells or bacteria:** Collect 400-500 × 10⁴ cells or bacteria into a centrifuge tube and discard the supernatant. Add 1 mL Reagent I, then sonicate for lysis for 1 min at 20% power using a cycle of 2 s sonication and 1 s pause. Use this as the test sample.

3. **Serum (plasma) and other samples:** Measure directly.

2. Reagent Preparation

Before use, prepare the TC working solution by pipetting about 0.8 mL of Reagent II and adding it separately to the Reagent III and Reagent IV bottles. Dissolve completely, then transfer all contents back into the Reagent II bottle and mix thoroughly. Incubate the TC working solution in a 37 °C water bath for 10 min before use.

Unused working solution should be stored at 4 °C for one month.

3. Procedure

1. Preheat the spectrophotometer for 30 min or longer, set the wavelength to 500 nm, and zero the instrument with distilled water.

2. Add reagents to EP tubes according to the table below.

Component	Standard Tube	Assay Tube	Blank Tube
TC standard (μL)	100	-	-
Sample solution (μL)	-	100	-
Reagent I (μL)	-	-	100
Working solution (μL)	900	900	900

3. Mix thoroughly, incubate at 37 °C for 10 min, then measure the absorbance at 500 nm. Record the values as A_{standard}, A_{assay}, and A_{blank}. The standard tube and blank tube only need to be measured once.

4. Total Cholesterol Content Calculation

4.1 Serum (plasma): TC (μmol/dL) = C_{standard} × (A_{assay} - A_{blank}) ÷ (A_{standard} - A_{blank}) × 100 mL = 50 × (A_{assay} - A_{blank}) ÷ (A_{standard} - A_{blank})

4.2 By sample protein concentration: TC (μmol/mg prot) = C_{standard} × (A_{assay} - A_{blank}) ÷ (A_{standard} - A_{blank}) ÷ C_{pr} = 0.5 × (A_{assay} - A_{blank}) ÷ (A_{standard} - A_{blank}) ÷ C_{pr}

4.3 By sample mass: TC (μmol/g fresh weight) = C_{standard} × (A_{assay} - A_{blank}) ÷ (A_{standard} - A_{blank}) ÷ W = 0.5 × (A_{assay} - A_{blank}) ÷ (A_{standard} - A_{blank}) ÷ W

4.4 In cells and bacteria: TC (μmol/10⁴ cells) = C_{standard} × (A_{assay} - A_{blank}) ÷ (A_{standard} - A_{blank}) ÷ bacteria or cells (10⁴ cells/L) = 0.5 × (A_{assay} - A_{blank}) ÷ (A_{standard} - A_{blank}) ÷ bacteria or cells (10⁴ cells/L)

C_{standard}: 0.5 μmol/mL

100 mL: 1 dL = 100 mL

C_{pr}: sample protein concentration, mg/mL

W: sample mass, g/mL

Precautions

1. Before formal testing, select 2-3 samples with large expected differences for preliminary testing.

2. Required instruments and supplies not provided with the kit: visible spectrophotometer, benchtop centrifuge, adjustable-volume pipette, 1 mL glass cuvette, mortar, ice, isopropanol, and distilled water.
3. This 50T kit can test 48 samples.
4. The minimum detection limit is 1 nmol/mL.

Visual Reference