# Free Cholesterol(FC) Content Assay Kit

Product Code: 112466

**112466 Free Cholesterol (FC) Content Assay Kit - Spectrophotometric Method**

## Product Introduction

Free cholesterol (FC) is a main component of cell membranes and a key precursor of adrenocortical hormones, sex hormones, bile acids, vitamin D, and other physiologically active substances. FC concentration can be used as an indicator of lipid metabolism.

FC oxidase catalyzes FC to generate Δ4-cholestenone and $H_2O_2$. Peroxidase then catalyzes $H_2O_2$, 4-aminoantipyrine, and phenol to form a red quinone compound with an absorption peak at 500 nm. The color intensity is directly proportional to the FC content.

This 50T kit can test 48 samples.

## Package Contents

| Pack Size | Code | Item | Quantity |
|---|---|---|---|
| 50T | 112466.1 | Reagent I (empty bottle) | 1 pc |
| 50T | 112466.2 | Reagent II | 1 bottle |
| 50T | 112466.3 | Reagent III | 1 bottle |
| 50T | 112466.4 | Reagent IV | 1 bottle |
| 50T | 112466.5 | Standard | 1 vial |
| 50T | 112466.m | Instructions | 1 copy |

Prepare isopropanol separately: 50 mL, designated as Reagent I.

## Quality Standards and Safety Instructions

| Raw Material | Quality Standards | Main Toxicity |
|---|---|---|
| Reagent II | - | - |
| Reagent III | - | - |
| Reagent IV | - | - |
| Standard | - | - |

## Transportation and Storage

**Transportation:** This product is shipped with ice packs.

**Storage:** Store at 2-8°C. Shelf life: 180 days.

## Instructions for Use

### 1. Free Cholesterol Extraction

1. **Tissue:** According to tissue mass (g), use a Reagent I volume (mL) to tissue mass ratio of 1:5-10. It is recommended to weigh about 0.1 g tissue and add 1 mL Reagent I for ice-bath homogenization. Centrifuge at 8000 g at 4°C for 10 min, collect the supernatant, and keep it on ice for testing.
2. **Bacteria and fungi:** Collect 4-5 million cells or bacteria into a centrifuge tube and discard the supernatant. Add 1 mL Reagent I and sonicate for 1 min at 20% power, using 2 s sonication and 1 s pause cycles. This is the FC test solution.
3. **Serum (plasma):** Measure directly.

## 2. Assay Procedure

1. Preheat the spectrophotometer for 30 min, set the wavelength to 500 nm, and zero with distilled water.
2. Prepare the working solution before use. Pipette approximately 0.8 mL of Reagent II into the Reagent III and Reagent IV bottles. After complete dissolution, transfer all contents back into the Reagent II bottle, mix thoroughly, and incubate in a 37°C water bath for 10 min. Unused working solution can be stored at 4°C for one week.
3. **Standard tube:** In a 1 mL glass cuvette, add 250 μL of standard and 750 μL of working solution. Mix well and let stand for 24 h, then measure the absorbance at 500 nm and record as $A_{standard}$. The standard tube only needs to be measured once.
4. **Assay tube:** In a 1 mL glass cuvette, add 250 μL of sample solution and 750 μL of working solution. Mix well and let stand for 24 h, then measure the absorbance at 500 nm and record as $A_{assay}$.

## 3. Calculation of Free Cholesterol Content

**3.1 Serum (plasma):** FC content (μmol/dL) = $C_{standard} \times A_{assay} \div A_{standard} \times 100\ mL = 50 \times A_{assay} \div A_{standard}$

**3.2 Based on sample protein concentration:** FC content (μmol/mg, prot) = $C_{standard} \times A_{assay} \div A_{standard} \div Cpr = 0.5 \times A_{assay} \div A_{standard} \div Cpr$

**3.3 Based on sample fresh weight:** FC content (μmol/g, fresh weight) = $C_{standard} \times A_{assay} \div A_{standard} \div W = 0.5 \times A_{assay} \div A_{standard} \div W$

**3.4 Cells and bacteria:** FC content (μmol/$10^4$cells) = $C_{standard} \times A_{assay} \div A_{standard} \div$ bacteria or cells ($10^4$cells/L) = $0.5 \times A_{assay} \div A_{standard} \div$ bacteria or cells ($10^4$cells/L)

$C_{standard}$: 0.5 μmol/mL

100 mL: 1 dL = 100 mL

Cpr: sample protein concentration, mg/mL

W: sample mass, g/mL

# Notes

1. After standing for 24 h, some samples may precipitate. Centrifuge at 10000 g, 25°C for 5 min before testing again.
2. The minimum detection limit is 1 nmol/mL.