Free Cholesterol (FC) Colorimetric Assay Kit

Cat #: orb1173224 (manual)

Product name: Free Cholesterol (FC) Colorimetric Assay Kit

Catalog number: orb1173224

Applicable samples: Serum, Plasma, Animal Tissues, Cells, Bacteria

Storage: Stored at -20℃ for 6 months, protected from light

Assay Principle

Free cholesterol (FC) is the main component of cell membranes and an important raw material for the synthesis of physiologically active substances such as adrenal cortex hormones, sex hormones, bile acids and vitamin D. Tissue FC concentration can be used as an indicator of lipid metabolism. Free Cholesterol (FC) Assay Kit provides a simple assay for the detection of FC in biological samples such as animal tissues, cells, bacteria, serum and plasma. The principle is that cholesterol oxidase catalyzes FC to generate Δ4-cholestenone and H2O2, and peroxidase catalyzes H2O2, 4-aminoantipyrine and phenol to generate red quinone compounds, with an absorption peak at 500 nm, the color depth is proportional to the FC content.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent II	5 mL	10 mL	4℃
Reagent III	10 mL	20 mL	4℃, protected from light
Standard	1 mL	1 mL	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 505 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated centrifuge, water bath
- Anhydrous ethanol
- Homogenizer (for tissue samples)

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Reagent Preparation

Working Reagent I: Prepared before use. Add 4 mL Reagent II for 48 T and 8 mL Reagent II for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent II: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Solution: Prepared before use, each well requires 200 µL of Working Reagent. Mix Working Reagent I and Reagent III in a ratio of 1:3. Working Solution is freshly prepared.

Standard: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal tissues: Weigh about 0.1 g tissues and add 1 mL anhydrous ethanol. Homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, discard the supernatant after centrifugation; add 1 mL anhydrous ethanol to ultrasonically disrupt the cells or bacteria in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum (plasma) samples: Directly test.

Note: The extraction buffer contains components that denature the protein. If the protein concentration is calculated, the protein needs to be extracted with deionized water for determination. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 500 nm, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (µL)	Standard Well (µL)	Test Well (µL)
Anhydrous Ethanol	5	0	0
Standard	0	5	0
Sample	0	0	5
Working Solution	200	200	200

Mix well and then incubate for 30 min at 37℃. Then reading the values at 505 nm. The absorbance of blank well, standard well, test well recorded as A_{Blank}, $A_{Standard}$ and A_{Test}. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be

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appropriately diluted with anhydrous ethanol, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. The test time should not exceed 1 h.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculate the content of TC in sample

(1) By sample fresh weight

FC (μmol/g)=$C_{Standard}\times\triangle A_{Test}\div\triangle A_{Standard}\times V_{Sample}\div(W\times V_{Test}\div V_{Sample\ Total})$**=1.25×$\triangle A_{Test}\div\triangle A_{Standard}\div$W**

(2) Calculated by protein concentration

FC (μmol/mg prot)==$C_{Standard}\times\triangle A_{Test}\div\triangle A_{Standard}\times V_{Sample}\div(Cpr\times V_{Sample})$**=5×$\triangle A_{Test}\div\triangle A_{Standard}\div$Cpr**

(3) Calculated by cells or bacteria number

FC (μmol/10^4)=$C_{Standard}\times\triangle A_{Test}\div\triangle A_{Standard}\times V_{Sample}\div(500\times V_{Sample}\div V_{Sample\ Total})$**=0.01×$\triangle A_{Test}\div\triangle A_{Standard}$**

(4) Calculated by liquid volume

FC (μmol/mL)=$C_{Standard}\times\triangle A_{Test}\div\triangle A_{Standard}$**=5×$\triangle A_{Test}\div\triangle A_{Standard}$**

Where: $C_{Standard}$: 5 μmol/mL; Cpr: Sample protein concentration, mg/mL; V_{Sample}: Sample volume added to the reaction system, 0.01 mL; W: Sample weight, g; $V_{Sample\ Total}$: Sample Preparation of added Extraction Buffer volume, 1 mL; 500: Total number of cells or bacteria, 5×10^6.

Typical Data

Add 5 μL mouse serum, according to the procedure, calculated:

A_{Test}=0.178, A_{Blank}=0.048, $A_{Standard}$=0.582; $\triangle A_{Test}$=0.178-0.048=0.13, $\triangle A_{Standard}$=0.582-0.048=0.534

Calculation of FC concentration (μmol/mL)=5×$\triangle A_{Test}\div\triangle A_{Standard}$=5×0.13÷0.534=1.22 μmol/mL

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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