EZMeta™ Free Cholesterol (FC) Colorimetric Assay Kit

Cat #: D-AKC2210

Size: 48T / 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

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

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. EZMeta™ Free Cholesterol (FC) Colorimetric 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 H_2O_2, and peroxidase catalyzes H_2O_2, 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	
FC Working Solution	10 mL	20 mL	4°C
Standard	1 mL	1 mL	4°C, protected from light

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 500 nm

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·Ice maker, refrigerated centrifuge, water bath

·Anhydrous ethanol

·Homogenizer (for tissue samples)

Reagent Preparation

FC Working Solution: Ready to use as supplied. Equilibrate to 37°Cbefore use. Store at 4°C.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Standard Curve Setting: Dilute the 2 μmol/mL Standard with absolute ethanol to 1, 0.5, 0.25, 0.125, 0.063, 0.031 μmoL/mL as indicated in the table below.

Num.	Volume of Standard	Volume of Anhydrous Ethanol (μL)	The Concentration of Standard (μmol/mL)
Std.1	200 μL of Standard	0	2
Std.2	100 μL of Std.1 (2 μmol/mL)	100	1
Std.3	100 μL of Std.2 (1 μmol/mL)	100	0.5
Std.4	100 μL of Std.3 (0.5 μmol/mL)	100	0.25
Std.5	100 μL of Std.4 (0.25 μmol/mL)	100	0.125
Std.6	100 μL of Std.5 (0.125 μmol/mL)	100	0.063
Std.7	100 μL of Std.6 (0.063 μmol/mL)	100	0.031

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C 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°C. 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°C. Use supernatant for assay, and place it on ice to be tested.

3. Serum (plasma) samples: Directly test.

Note: It will be better to quantify the total protein with EZMeta™ Free Cholesterol (FC) Colorimetric Assay Kit, 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. Warm FC Working Solution to 37°C for more than 30 min before use.

3. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Anhydrous Ethanol	50	0	0
Standard	0	50	0
Sample	0	0	50
FC Working Solution	150	150	150

4. Mix well and then incubatefor 15 min at room temperature. Then reading the values at 500 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 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 2.0, the sample can be 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. Drawing of standard curve

With the concentration of the Standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

Substitute the ΔA_{Test} into the equation to obtain the y value (μmoL/mL).

2. Calculate the content of FC in sample

(1) By sample fresh weight

FC (μmol/g)= $y \div (W \div V_{Extraction}) \times n$**=y÷W×n**

(2) Calculated by protein concentration

FC (μmol/mg prot)**=y÷Cpr×n**

(3) Calculated by cells or bacteria number

FC (μmol/10^4)=$y \div$(cells or bacteria number$\div V_{Extraction}) \times n$**=y÷500×n**

(4) Calculated by liquid volume

FC (μmol/mL)**=y×n**

Where: W: sample weight, g; $V_{Extraction}$: Anhydrous ethanol volume added, 1 mL; n: dilution factor; Cpr: supernatant protein concentration, mg/mL; 500: Total number of cells or bacteria, 5×10^6.

Typical Data

Typical standard curve:

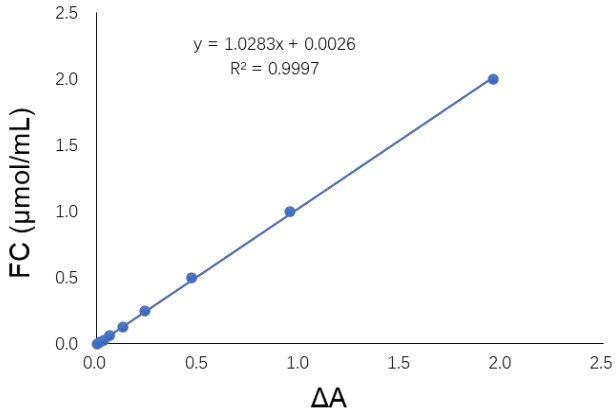

Figure1. Standard Curve for FC.

Examples

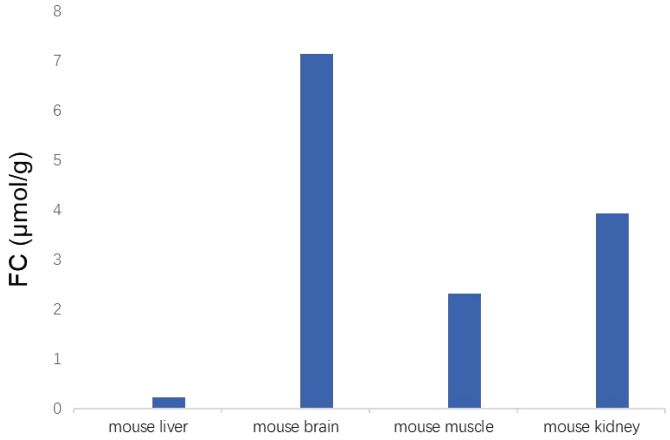

Figure 2. FC content in animal tissues. Assays were performed following kit protocol.

Disclaimer

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