Free Cholestenone (FC) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Cat No: BC1890

Size: 50T/48S

Components: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any doubts, please contact the Solarbio staff in time.

Reagent Name	Size	Storage
Extract solution	Self-Provided Reagent	-
Reagent I	75 mL×1	2-8℃
Reagent II	450 μL×1	2-8℃
Standard	Poeder×1 支	2-8℃

Extract solution: Isopropanol (required but not provided. It takes about 60mL), store at RT. A 30 mL brown empty bottle is provided in the kit, which is only used for packaging. Please mark the name of the reagent yourself.

Standard: Powder×1, 10 mg cholesterol, store at 2-8℃. Add 517 μL isopropanol and prepare as 50 μmol/mL cholesterol standard solution, then diluted to 1 μmol/mL standard with isopropanol for test. The unused reagent can be stored at 2-8℃ for 4 weeks.

Working solution: According to the sample number, the working solution is prepared according to the ratio of Reagent 1 : Reagent 2 =9mL : 60μL (about 10T).

Description:

FC is the main component of cell membrane, and it is also an important raw material for the synthesis of adrenocortical hormone, sex hormone, bile acid and vitamin D. The concentration of FC can be used as an index of lipid metabolism. The determination principle: FC oxidase catalyzes FC to form 4-cholesterolenone and H_2O_2, while the peroxidase catalyzes H_2O_2, 4-aminoantipyrine and phenol to form red quinone compounds with an absorption peak at 500 nm, and the color depth is proportional to the content of FC.

Required but not provided:

Water bath, pipettes, spectrophotometer, 1 mL glass cuvette, mortar/homogenizer/cell ultrasonic crusher, absolute ethanol, distilled water.

Protocol:

I. Sample Preparation.

1. Tissue:

Accordance ratio weight (g): Extract solution(mL)=1: 5~10. (Add 1 mL Extract solution to 0.1 g tissue). Homogenate on ice bath. Centrifuge at 8000 g for 10 min at 4℃. Take supernatant for test.

2. Bacteria or fungus:

Accordance ratio cell amount (10^4): Extract solution(mL)=500~1000:1. (Suggest 5 million with 1 mL Extract solution). Break cells (power 300w, work time 2s, interval 3s for 3 min) by ultrasonic on ice bath. 8000 g centrifuge for 10 min at 4℃. Take supernatant for test.

3. Serum (plasma) sample: Detect directly.

II. Determination procedure.

1. Preheat spectrophotometer for 30 min, adjust wavelength to 500 nm, set zero with distilled water.
2. Take out certain quantity working solution, preheat at 37℃ for 30 min. The rest store at 2-8℃.
3. Dilution of standard solution: 50μmol/mL cholesterol standard solution is diluted with extract solution to obtain 1.25, 0.625, 0.3125, 0.15625, 0.078125, 0.039, 0.0195μmol/mL standard substance for later use.
4. Standard dilution can refer to the following table：

-	Pre-dilution concentration（μmol/mL）	standard solution volume（μL）	extract solution volume（μL）	Concentration after dilution（μmol/mL）
1	50	25	975	1.25
2	1.25	500	500	0.625
3	0.625	500	500	0.3125
4	0.3125	500	500	0.15625
5	0.15625	500	500	0.078125
6	0.078125	500	500	0.039
7	0.039	500	500	0.0195

5. Add reagents according to the following table.

Reagent Name:	Blank tube (A_B)	Standard tube (A_S)	Test tube (A_T)
Sample	100	-	-
Standard	-	100	-
Isopropanol	-	-	100
Working solution	900	900	900

Fully mixed, stand at 37 °C for 30 min. After the reaction completed, the absorbance value A at 500 nm is measured in 1 mL glass cuvette, which is recorded as A_T, A_S and A_B, $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$. Blank tube and standard curve only need to measure 1-2 times.

Note: If the sample is a liquid sample such as serum (plasma), it is necessary to add a ' serum (plasma) blank tube ' -the extract (isopropanol) in the blank tube is replaced with distilled water for the experiment, and the calculation of $\Delta A_T = A_T - A_{B\text{-serum (plasma)}}$, standard tube determination and ΔA_S calculation remain unchanged.

III. Calculation.

1. Standard curve

According to the concentration of the standard tube (x，μmol/mL) and the absorbance ΔAs (y, ΔAs), a standard curve was established. According to the standard curve, ΔA (y, ΔA) was brought into the formula to calculate the sample concentration (x，μmol/mL).

2. Calculate of TC content

(1) Serum (plasma)

TC centent (μmol/dL) =x×100

(2) Tissue

a. Calculate by protein concentration

TC content (μmol/mg prot) =x×VE÷(Cpr×VE)=x÷Cpr

b. Calculate by sample weight

TC content (μmol/g fresh weight) =x×VE÷W=x÷W

(3) Cells

TC content (μmol/104 cell)=x×VE÷500=0.002x

100:1 dL=100 mL

VE: Extract volume, 1 mL;

W: Sample weight, g;

500: The number of cells, 500 million;

Cpr: The concentration of protein, mg/mL.

Note:

1. If the measured absorbance value exceeds the linear range absorbance value, you can increase the sample size or dilute the sample with the extraction solution and then perform the measurement. Note the simultaneous modification of the calculation formula.
2. The extraction solution contains components that denature the protein, so it is necessary to re-extract the protein for measurement when calculating by protein concentration.

Technical Specifications：

Minimum Detection Limit：0.056 μmol/mL

Linear Range：0.078-2 μmol/mL

Recent Product citations：

[1] Qin Yuan,Shang Lin,Yuan Fu,et al. Effects of extraction methods on the physicochemical characteristics and biological activities of polysaccharides from okra (Abelmoschus esculentus). International Journal of Biological Macromolecules. April 2019;127:178-186.(IF4.784)

[2] Huan Guo,Shang Lin,MinLua Jia,et al. Characterization, in vitro binding properties, and inhibitory activity on pancreatic lipase of β-glucans from different Qingke (Tibetan hulless barley) cultivars. International Journal of Biological Macromolecules. December 2018;(IF4.784)

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[3] Yao L, Chen S, Li W. Fatostatin inhibits the development of endometrial carcinoma in endometrial carcinoma cells and a xenograft model by targeting lipid metabolism[J]. Archives of Biochemistry and Biophysics, 2020: 108327.

Related products:

BC0590/BC0595	Free fatty Acids(FFA) Assay Kit
BC2340/BC2345	Lipase(LPS) Activity Assay Kit
BC1080/BC1085	Alcohol dehydrogenase (ADH) Assay Kit
BC1070/BC1075	Pyruvate Decarboxylase (PDC) Assay Kit
BC0620/BC0625	Triglyceride(TG) Assay Kit

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