Free Cholestenone (FC) Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC1895

Size: 100T/96S

Components:

Extract solution: Isopropanol (required but not provided, It takes about 110mL), 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.

Reagent I: Liquid 30 mL×1, store at 2-8℃.

Reagent II: Liquid 160μL×1, store at 2-8℃.

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 2 μmol/mL standard with isopropanol for test. The unused reagent can be stored at 2-8℃ for 4 weeks.

Working Solution preparation: According to the sample amount, reagent I : reagent II is prepared in a ratio of 3 mL : 20 μL (about 16 T).

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/microplate reader, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, isopropanol and distilled water.

Protocol:

I. Sample Preparation.

1. Tissue:

Accordance ratio weight(g): Extract solution(mL)=1: 5~10. (Suggested 0.1 g tissue with 1 mL Extract solution). Homogenate on ice bath. Centrifuge at 8000 g for 10 min at 4℃. Supernatant is for test on ice.

2. Bacteria or fungus:

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

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3. Serum (plasma) sample: Detect directly.

II. Determination procedure.

1. Preheat spectrophotometer/microplate reader for 30 min, adjust wavelength to 500 nm, set zero with
distilled water.
2. Preheat working solution at 37°C for 10 min.
3. Dilution of standard: 50μmol/mL cholesterol standard substance is diluted with Extract solution to obtain 2.5, 2, 1.25, 0.625, 0.3125, 0.15625 μmol/mL standard for later use.
4. Add reagents according to the following table.

Reagent Name（μL）	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	20	-	-
Standard	-	20	-
Extract solution	-	-	20
Working solution	180	180	180

Mix thoroughly, detect absorbance at 500 nm after incubating at room temperature for 30 min. Record A_B, A_S, A_T. The standard curve and blank tube only need to be measured 1-2 times.

Note: If the sample is 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 $\Delta A_T = A_T - A_{\text{serum (plasma) blank}}$, standard tube determination and ΔA_S calculation remain unchanged.

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) is brought into the formula to calculate the sample concentration (x，μmol/mL).

2. Calculate of FC content

(1) Serum (plasma)

FC centent (μmol/dL) =x×100×F

(2) Tissue

a. Calculate by protein concentration

FC content (μmol/mg prot) =$x \times V_E \div (Cpr \times V_E) \times F = x \div Cpr \times F$

b. Calculate by sample weight

FC content (μmol/g fresh weight) ×F =$x \times V_E \div W = x \div W \times F$

(3) Cells

FC content (μmol/10^4 cell)=$x \times V_E \div N \times F = 0.002x \times F$

100:1 dL=100 mL

V_E: Extract volume, 1 mL;

W: Sample weight, g;

N: The number of cells, 10^4 cell as a unit;

Cpr: The concentration of protein, mg/mL;

F: dilution multiple.

Recent Product citations:

[1] Wang P, Li M, Gao T, Fan J, Zhang D, Zhao Y, Zhao Y, Wang Y, Guo T, Gao X, Liu Y, Gao Y, Guan X, Sun X, Zhao J, Li H, Yang L. Vascular Electrical Stimulation with Wireless, Battery-Free, and Fully Implantable Features Reduces Atherosclerotic Plaque Formation Through Sirt1-Mediated Autophagy. Small. 2023 Oct;19(40):e2300584. doi: 10.1002/smll.202300584. Epub 2023 Jun 2. PMID: 37267941.

[2] Zhao C, Li L, Li C, Tang C, Cai J, Liu Y, Yang J, Xi Y, Yang M, Jiang N, Han Y, Liu Y, Luo S, Xiao L, Sun L. PACS-2 deficiency in tubular cells aggravates lipid-related kidney injury in diabetic kidney disease. Mol Med. 2022 Sep 23;28(1):117. doi: 10.1186/s10020-022-00545-x. PMID: 36138342; PMCID: PMC9502582.

[3] Ye L, Zhu M, Ju J, Yang H. Effects of Dietary Cholesterol Regulation on Spermatogenesis of Gobiocypris rarus Rare Minnow. Int J Mol Sci. 2023 Apr 19;24(8):7492. doi: 10.3390/ijms24087492. PMID: 37108655; PMCID: PMC10141657.

[4] Li RX, Chen LY, Limbu SM, Qian YC, Zhou WH, Chen LQ, Luo Y, Qiao F, Zhang ML, Du ZY. High cholesterol intake remodels cholesterol turnover and energy homeostasis in Nile tilapia (Oreochromis niloticus). Mar Life Sci Technol. 2023 Feb 16;5(1):56-74. doi: 10.1007/s42995-022-00158-7. PMID: 37073330; PMCID: PMC10077235.

[5] Deng WY, Zhou CL, Zeng MY. Gypenoside XVII inhibits ox-LDL-induced macrophage inflammatory responses and promotes cholesterol efflux through activating the miR-182-5p/HDAC9 signaling pathway. J Ethnopharmacol. 2024 Jan 30;319(Pt 1):117070. doi: 10.1016/j.jep.2023.117070. Epub 2023 Aug 23. PMID: 37625608.

reference:

[1] Lie RF, Schmitz JM, Pierre KJ. et al. Cholesterol oxidase-based determination, by continuous-flow analysis, of total and free cholesterol in serum[J]. Clinical Chemistry, 1976, 22(10): 1627-1630.

[2] Otani T, Ishimaru K, Nakamura S. et al. Determination of total and free cholesterol by using cholesterol oxidase from Streptomyces [J]. Chemical & pharmaceutical bulletin, 1977, 25(6): 1452-1455.

Related products:

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BC1080/BC1085 Alcohol dehydrogenase (ADH) Assay Kit

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