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Free Cholestenone(FC) Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: AK0411

Size: 100T/96S

Components:

Extract solution: Isopropanol 100 mL (required but not provided), store at RT.

Working solution: Liquid 20 mL×1, store at 4℃ .

Standard: Powder×1, 10 mg cholesterol, store at 4℃ . Add 517 μL isopropanol and prepare as 50 μmol/mL cholesterol standard solution, then diluted to 2 μmol/mL standard with isopropanol for test.

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, absolute ethanol 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.

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.

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. Take out certain quantity working solution, preheat at 37℃ for 30 min. The rest store at 4℃ .

3. Add reagents according to the following table.

(μL)/tube	Isopropanol	Standard	Supernatant	Working solution
Blank tube (A_B)	10			190
Standard tube (A_S)		10		190
Test tube (A_T)			10	190

Mix thoroughly, detect absorbance at 500 nm after incubating at room temperature for 15 min. Record A_B, A_S, A_T . Only 1-2 blank tubes and standard tubes are required.

III. Calculation.

1. Serum (plasma) sample

$FC(\mu mol/dL)= C_S \times(A_T-A_B)\div(A_S-A_B)\times100=200\times(A_T-A_B)\div(A_S-A_B)$

C_S : Standard concentration, 2 μmol/mL;

100: Unit conversion factor, 1 dL = 100 mL.

2. Tissue

A. Protein concentration:

$FC\ (\mu mol/mg\ prot)= C_S \times(A_T-A_B)\div(A_S-A_B)\times V_{ST}\div(Cpr\times V_{ST})=2\times(A_T-A_B)\div(A_S-A_B)\div Cpr$

B. Sample weight

$FC\ (\mu mol/g\ weight)= C_S \times(A_T-A_B)\div(A_S-A_B)\times V_{ST}\div W =2\times(A_T-A_B)\div(A_S-A_B)\div W$

C_S : Standard concentration, 2 μmol/mL;

V_{ST} : Extract solution volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g.

3. Cells or bacteria

FC (μmol/10^4cell)=$C_S \times (A_T-A_B) \div (A_S-A_B) \times V_{ST} \div 500 = 0.004 \times (A_T-A_B) \div (A_S-A_B)$

C_S : Standard concentration, 2 μmol/mL;

500: The amount of Cells or bacteria, 5 million;

$V_{ST:}$ The volume of extract solution, 1 mL.

Technical Specifications：

Minimum Detection Limit：0.119 μmol/mL

Linear Range ：0. 125-6 μmol/mL

Experimental example:

1. Take 0. 1g mouse liver to 1ml extract solution, grinding and operate as the procedure after taking the supernatant, A_B=0.046, A_T=0. 127, A_S=0.354, calculate content by sample weight: FC（μmol/g weight)= $(A_T-A_B) \div (A_S-A_B) \div W$=5.320 μmol/g weight.

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)

[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.

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