Total cholesterol (TC) Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC1985

Size: 100T/96S

Components:

Extract: Isopropyl alcohol 100 mL ×1. Required but not provided. Store at 2-8℃. 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 160 μL×1. Store at 2-8℃.

Reagent III: Liquid 25 μL×1. Store at 2-8℃. The liquid is placed in an EP tube inside the reagent bottle.

Standard solution: Powder ×1. Store at 2-8℃. 10 mg cholesterol, add 517 μL extract before use and shake to dissolve, 50 μmol/mL cholesterol standard solution, the reagent can be stored at 2-8℃ for 4 weeks.

Working solution: Prepare the working solution according to the sample volume of Reagent I: Reagent II: Reagent III in the ratio of 3mL: 20μL: 3μL (about 16T),when the solution will be used.

Product Description

Total cholesterol (TC) is the sum of cholesterol contained in all lipoproteins, including free cholesterol and cholesteryl esters.

The enzyme esterase catalyzes the hydrolysis of cholesteryl esters to produce free cholesterol (FC) and free fatty acids (FFA), thus converting cholesteryl esters to FC; furthermore, cholesteryl oxidase catalyzes the oxidation of FC to produce 4-Cholestenone and H2O2; finally, peroxidase catalyzes the oxidation of 4-aminoantipyrine and phenol by H2O2 to produce red quinones, which have a characteristic absorption peak at 500 nm. The color shade is proportional to the TC content.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, balance, low temperature table centrifuge, water-bath, micro glass cuvette/96 well flat-bottom plate, pipette, EP tube, distilled water, isopropyl alcohol.

Procedure

I. Crude enzyme extraction :

1. Tissue: according to the tissue weight (g) : the extract volume (mL) is 1:5-10. (It is recommended that add 1 mL of extract to 0.1 g tissue). Homogenate in ice bath, then centrifuge at 10000 g for 10 minutes at 4℃. Take the supernatant for test.

2. Cells: according to the number of the cells (10^4): the volume of the extract (mL) is 500~1000:1. It is suggest that add 1 mL of Extract to 500 million of cells. Breaking cells by ultrasonic wave

in ice bath (power 300W, ultrasonic 2s, interval 3s, total time 3 min). Centrifuge at 10000 g 4℃ for 10 minutes. Take the supernatant on ice for test.

3. Serum (plasma) or urine: detect directly. If the solution has precipitate, please centrifuge and take the supernatant to be measured

II. Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 500 nm and the spectrophotometer set the counter to zero with distilled water.

2. Dilute 50 μmol/mL standard solution with distilled water to 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078 μmol/mL for standby.

3. Operation table: (Add the following reagents into 1.5 mL centrifuge tube/96 well flat-bottom plate)

Reagent Name (μL)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	20	-	-
Standard	-	20	-
Extract	-	-	20
Working solution	180	180	180
Mix thoroughly. React in 37℃ water bath or constant temperature incubator for 15 min. Use micro glass cuvette/96 well flat-bottom plate to measure the absorption value A at 500 nm. Record as A_T, A_S, A_B. $\Delta A=A_T-A_B$. $\Delta A_S=As-A_B$. Each blank tube only needs to test once or twice. **Note:** If the sample is serum, it is necessary to fill the serum blank tube (A_B'): change the extract solution (isopropanol) to distilled water for the experiment. Calculate $A=A_T-A_B$'. Standard tube and Blank tube 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) 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: 0.143 μmol/mL

Linear Range: 0.156-5 μmol/mL

Experimental example:

1. Take rabbit serum, operate as the procedure, $\Delta A=A_T-A_B=0.092-0.048=0.044$, standard curve: y =0.4331x-0.0623, x=0.2454, calculate content by plasma volumn:
   TC（μmol/dL)= x×100=0.2454×100=24.54 μmol/dL.

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]Wei Hu, Rui Wei, LiYue Wang,et al. Correlations of MMP-1, MMP-3, and MMP-12 with the degree of atherosclerosis, plaque stability and cardiovascular and cerebrovascular events. EXPERIMENTAL AND THERAPEUTIC MEDICINE. 2018;(IF1.448)

[3]Jieyong Xing,Yanshao Liu,Tao Chen,et al. Correlations of chemokine CXCL16 and TNF-α with coronary atherosclerotic heart disease. Experimental and Therapeutic Medicine. November 2017;(IF1.448)

[4]Jiabin Huang,Shangjun Chen,Dongliang,et al. Long noncoding RNA lncARSR promotes hepatic cholesterol biosynthesis via modulating Akt/SREBP-2/HMGCR pathway. Life Sciences. June 2018;(IF3.448)

[5]Li W, Li Y, Zhao Y, et al. The protective effects of aloperine against ox-LDL-induced endothelial dysfunction and inflammation in HUVECs[J]. Artificial Cells, Nanomedicine, and Biotechnology, 2020, 48(1): 107-115.

Related products:

BC1890/BC1895	Free Cholestenone(FC) Content Assay Kit
BC0590/BC0595	Free fatty Acids(FFA) Content Assay Kit
BC0750/BC0755	Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit

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