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Total cholesterol (TC) Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0327

Size: 50T/48S

Components:

Extract: Isopropyl alcohol 50 mL ×1. Required but not provided. Store at 4℃.

Reagent I: Liquid 60 mL×1. Store at 4℃.

Reagent Ⅱ: Liquid 400 μL×1. Store at 4℃.

Reagent Ⅲ: Liquid 60 μL×1. Store at 4℃.

Standard solution : Powder ×1 bottle, 10 mg cholesterol. Store at 4℃. Add 517 μL of Extract before use, and shake to dissolve. It is prepared into a cholesterol standard solution of 50 μmol/mL.

Working solution: According to the ratio of Reagent I: Reagent Ⅱ: Reagent Ⅲ=3 mL: 20 μL: 3 μL to prepare when the solution will be used.

Product Description

Total cholesterol (TC) refers to the total cholesterol of all lipoproteins. It includes free cholesterol and cholesterol esters.

Esterase can catalyze the hydrolysis of cholesterol ester to produce free cholesterol (FC) and free fatty acid (FFA), thus transforming cholesterol ester into FC; Furthermore, cholesterol oxidase can catalyze FC to form Δ4-cholesterone and H_2O_2 ; Finally, peroxidase can catalyze the oxidation of 4-aminoantipyrine and phenol by H_2O_2 to form red quinones. It has a characteristic absorption peak at 500 nm, and its color depth is directly proportional to TC content.

Reagents and Equipment Required but Not Provided.

Spectrophotometer, balance, low temperature table centrifuge, water-bath, 1 mL glass cuvette, pipette, EP tube, distilled water, isopropyl alcohol.

Procedure

I. Crude enzyme extraction :

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1. Tissue: according to the tissue weight (g) : the Extract liquid 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.

II. Determination Procedure

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

2. Dilute 50 μmol/mL standard solution with distilled water to 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)

Reagent Name (μL)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	100	-	-
Standard	-	100	-
Extract	-	-	100
Working solution	900	900	900
Mix thoroughly. React in 37℃ water bath or constant temperature incubator for 15 min. Use 1 mL glass cuvette 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.			

III. Calculation of UA:

1. Standard curve

Take the concentration of each standard solution as x-axis, and the corresponding ΔA standard is y-axis. Then the linear regression equation y＝kx+b is obtained. Bring ΔA into the equation to get 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) =$x \times V_E \div (Cpr \times V_E) = x \div Cpr$

b. Calculate by sample weight

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

3. Cells

TC content (μmol/10^4 cell)=$x \times V_E \div 500 = 0.002x$

100:1 dL=100 mL

V_E: Extract volume, 1 mL;

W: Sample weight, g;

500: The number of cells, 500 million;

Cpr: The concentration of protein, mg/mL.

Note:

1. When ΔA is more than 1, diluted the sample with extract and then determined.

2. The protein concentration can be detected in another tissue.

Technical Specifications：

Minimum Detection Limit：0.056 μmol/mL

Linear Range ：0.078-2 μmol/mL

Experimental example:

Take chicken plasma, operate as the procedure, $\Delta A = A_T - A_B = 0.083 - 0.012 = 0.071$, standard curve: y =0.6686x-0.035, x=0. 1585, calculate content by plasma volumn: TC（μmol/dL)= x × 100=0. 1585× 100=15.85 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：

AK0412/AK0411 Free Cholestenone(FC) Content Assay Kit

AK0536/AK0535 Free fatty Acids(FFA) Content Assay Kit

AK0269/AK0268 Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit