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High-Density Lipoprotein Cholesterol (HDL-C) Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: AK0693-100T/96S

Size: 100T/96S

Components:

Extract I: Isopropyl alcohol 110 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.

Extract ⅡA: Liquid 6 mL×1. Store at 2-8℃.

Extract ⅡB: Liquid 6 mL×1. Store at 2-8℃. According to the required amount of the experiment, according to the ratio of extract ⅡA: extract ⅡB =50 μL: 50 μL (1T amount), mix well, and prepare before use.

Reagent IA: Powder ×2. Store at 2-8℃.

Reagent IB: Liquid 30 mL×1. Store at 2-8℃. Add 14mL of Reagent IB to one Reagent IA and shake to dissolve. It could be stored at 2-8℃ for two weeks.

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

Reagent Ⅲ: Liquid 25 μL×1. Store at 2-8℃.

Standard Solution: Powder ×1, 10 mg cholesterol. Store at 2-8℃. Add 517 μL of Extract I before use and shake to dissolve. The cholesterol standard solution of 50 μmol/mL could be stored at 2-8℃ for four weeks.

Working Solution: According to the required amount of the experiment, according to the ratio of Reagent I: Reagent Ⅱ: Reagent Ⅲ=3mL: 20 μL: 3 μL (16T amount), mix well, and prepare before use

Product Description

High-density lipoprotein (HDL) is a lipoprotein that contains a small amount of cholesterol and carries cholesterol away from body cells and tissues. High-density lipoprotein cholesterol (HDL-C) concentrations negatively correlate with the incidence of atherosclerosis and coronary heart disease. Therefore, accurate and precise measurements of patients' HDL-C concentrations are necessary to appropriately identify individuals with atherosclerosis, coronary heart disease and hypertension.

Cholesterol is specifically dissociated by one surfactant from HDL. 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 phenyl

amines by H_2O_2 to form purple quinones. It has a characteristic absorption peak at 546 nm, and its color depth is directly proportional to cholesterol content.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, balance, low temperature table centrifuge, constant temperature incubator/water bath, micro glass cuvette/96 well flat-bottom plate, pipette, mortar/homogenizer/cell

ultrasonic crusher, ice, distilled water, **isopropyl alcohol**.

Procedure

I. Sample preparation:

1. Tissue: according to tissue weight (g): Extract I volume (mL) is 1:5-10. (It is recommended that add 1 mL of Extract I 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. Bacteria/cells: according to the number of bacteria/cells (10^4): the volume of Extract I (mL) is 500~1000:1. It is suggest that add 1 mL of Extract I to 500 million of cells. Breaking bacteria/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 other liquid samples: detect directly. Centrifuge before detecting if there are precipitation in the liquid.

II. Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 546 nm and set spectrophotometer counter to zero with distilled water.

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

3. Operation table:

Reagent (μL)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	100	-	-
Standard	-	100	-
Extract Ⅱ	100	100	-
Mix well. React at room temperature for 10 minutes. Centrifuge at 3000rpm for 15 minutes at room temperature and take the supernatant.			
Supernatant	20	20	-
Extract I	-	-	20
Working Solution	180	180	180
Mix thoroughly. React at 37℃ for one hour. Measure the absorption at 546 nm and record as A_T, A_S, A_B. Calculate $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$. Blank tube and standard curve only need to test once or twice.			

III. Calculation of HDL-C Content:

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation $y = kx + b$, and bring ΔA_T into the equation to get x (μmol/mL).

2. Calculation

1) Serum (plasma) or other liquid samples: HDL-C content (μmol/dL) =x×100
2) Protein concentration: HDL-C content (μmol/mg prot) =$x \times V_E \div (Cpr \times V_E)$ =x÷Cpr

3) Sample weight: HDL-C content (μmol/g weight) =$x \times V_E \div W$ =x÷W
4) Bacteria/cells number: HDL-C content (μmol/10^4 cell) =$x \times V_E \div 500$=0.002x

100: Unit conversion factor, 1 dL= 100 mL;
V_E: Added Extract I volume, 1 mL;
W: Sample weight, g;
500: The number of bacteria/cells, 500 million;
Cpr: The concentration of protein, mg/mL;

Note:

1. If samples ΔA_T is too high, it is suggested that the samples should be diluted with multiple times of Extract I. Sample supernatant volume could be increased if samples ΔA_T is too low. And modify the calculation formula.
2. The protein concentration can be detected in another tissue.

Experimental example:

1. Take 0.1 mL of human serum, operate according to the determination steps, calculate $\Delta A_T = A_T - A_B$ =0.142-0.060=0.082. Bring the result into the standard curve y=0.3888x-0.0502 and calculate x=0.340. The result is calculated according to liquid volume:
   HDL-C content (μmol/dL) =x×100=0.340× 100=34.002 μmol/dL.
2. Take 0.1g fish liver, add 1 mL of Extract I, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T = A_T - A_B$ =0.089-0.060=0.029. Bring the result into the standard curve y=0.3888x-0.0502 and calculate x=0.204. The result is calculated according to
   sample weight:
   HDL-C content (μmol/g weight) =x÷W =0.204÷0.1=2.037 μmol/g weight.