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Low-Density Lipoprotein Cholesterol (LDL-C) Content Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: AK0738-100T-96S

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent Name	Size	Preservation condition
Extract Solution	Self-Provided Reagent	-
Reagent I A	Liquid 25 mL×1	2-8℃
Reagent I B	Liquid 200 μL×1	2-8℃
Reagent I C	Liquid 30 μL×1	2-8℃
Reagent II	Liquid 8 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution reparation:

1. **Extract Solution I:** Prepare your own isopropyl alcohol, about 110mL, stored at room temperature; 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.
2. **Reagent I B:** Shake the liquid from the bottle to the bottom before use. (Use a handheld centrifuge)
3. **Reagent I C:** The liquid is placed in the EP tube inside the reagent bottle. Shake the liquid from the bottle to the bottom before use. (Use a handheld centrifuge)
4. **Reagent I:** Prepare the working solution according to the ratio of Reagent I A: Reagent I B: Reagent I C =2.25 mL:20 **μL**:3 **μL**(2.273mL, 12T) according to the sample volume, and prepare the working solution as it is used.
5. **Standard:** 10 mg cholesterol. Add 517 μL of Extract Solution I before use and shake to dissolve. The cholesterol standard solution of 50 μmol/mL could be stored at 2-8℃ for four weeks.

Product Description

Low density lipoprotein is a kind of lipoprotein with the highest cholesterol content in serum lipoprotein, and its particles are small. Its main role is to transport cholesterol from liver tissue to other tissues through the blood, and promote the accumulation of cholesterol in tissue cells. Many epidemiological studies have shown that serum low density lipoprotein cholesterol levels are positively correlated with atherosclerosis (AS) and coronary heart disease (GHD), which has important reference value for clinical diagnosis of atherosclerosis, coronary heart disease, hypertension and other diseases.

Cholesterol of chylomicrons (CM), very-low-density lipoproteins (VLDL), high-density lipoproteins (HDL) is specifically dissociated by one surfactant, but LDL-C is not dissociated by the surfactant. Cholesterol ester and cholesterol oxidase can catalyze the hydrolysis of dissociated cholesterol to produce H_2O_2, which cannot form colored compounds without chromogenic agents. Cholesterol is specifically dissociated by another surfactant from undissociated LDL. 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 4-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 plate, mortar/homogenizer/cell ultrasonic crusher, adjustable pipette, ice, distilled water, **isopropyl alcohol**(>98%，AR).

Operation procedure

I. Sample preparation: (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

1. **Tissue:** according to the tissue mass (g): the Extract Solution volume (mL) is 1:5-10. (It is recommended that add 1 mL of Extract Solution to 0.1 g tissue). Homogenate in ice bath, then centrifuge at 10000 g for 10 minutes at 4℃. Take the supernatant on ice and leave it to be measured.
2. **Bacteria/cells:** according to the number of bacteria/cells (10^6): the volume of Extract Solution (mL) is 5~10:1. It is recommended that add 1 mL of Extract Solution to 5 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 and leave it to be measured.
3. **Serum (plasma) or other liquid samples:** Directly measured. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 546 nm and set spectrophotometer to zero with distilled water.
2. Reagent I and Reagent II were taken according to the sample volume and preheated at 37°C for 10 min.
3. Standard working solution: Dilute 50 μmol/mL standard solution with **Extract Solution** to 2, 1.25, 0.625, 0.3125, 0.15625, 0.078125μmol/mL for standby.
4. Standard dilution table

Serial number	The concentration before dilution(μmol/mL)	Standard volume(μL)	Volume of **Extract Solution** (μL)	Diluted concentration (μmol/mL)
1	50	50	950	2.5

2	2.5	400	100	2
3	2.5	200	200	1.25
4	1.25	200	200	0.625
5	0.625	200	200	0.3125
6	0.3125	200	200	0.15625
7	0.15625	200	200	0.078125

Note: The following experiments are requiring 5μL of standard for each standard tube. (Be careful not to test the absorbance of the standard directly in this step.)

5. Operation table:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)	Standard tube (A_S)
Sample	5	-	-
Extract Solution	-	5	-
Reagent I	180	180	180
Mix well. React at 37℃ for 5 minutes. Measure the absorption at 546 nm and record as $A1_T$, $A1_B$.			
Reagent II	60	60	60
Standard			
Mix well. React at 37℃ for 5 minutes. Measure the absorption at 546 nm and record as $A2_T$, $A2_B$, A_S. Calculate $\Delta A_T = (A2_T - A1_T)- (A2_B - A1_B)$, $\Delta A_S = A_S - A2_B$. Blank tube and standard curve only need to test once or twice.			

Note: If the sample is a liquid sample such as serum (plasma), it is necessary to add a 'serum (plasma) blank tube' - i.e., the Extract Solution (isopropanol) in the blank tube is replaced with distilled water for the experiment, and the $\Delta A_T = A_T - A_B$(serum (plasma) blank), while the assay in the standard tube and the calculation of ΔA standard remain unchanged.

III. Calculation of LDL-C Content:

1. Standard curve

According to the concentration (x, μmol/mL) of the standard tube and the absorbance ΔAs (y, ΔAs), establish a standard curve. According to the standard curve, bring ΔA_T (y, ΔA_T) into the formula to calculate the sample concentration (x, μmol/mL).

2. Calculation

1) Serum (plasma) or other liquid samples: LDL-C content (μmol/dL) =x×100×F
2) Protein concentration: LDL-C content (μmol/mg prot) =$x \times V_S \div (Cpr \times V_S) \times F = x \div Cpr \times F$
3) Sample mass: LDL-C content (μmol/g mass) =$x \times Vs \div (W \times Vs \div V_E) \times F = x \div W \times F$
4) Bacteria/cells number: LDL-C content (nmol/10^6 cell) =$x \times Vs \div (N \times Vs \div V_E) \times F = x \div N \times F$

100: Unit conversion factor, 1 dL=100 mL;V_S: Added sample volume, 0.005 mL; V_E: Extract Solution volume, 1 mL; W: Sample mass, g; N: The number of bacteria/cells, $\times 10^6$;Cpr: The concentration of

protein, mg/mL;F: Dilution multiple.

Note:

1. If the measured absorbance value is below or above the linear range absorbance value, the sample size can be increased or the sample can be diluted with the Extract Solution (the liquid sample is diluted with distilled water) before the determination. Change the calculation formula simultaneously.
2. The Extract Solution contains components that denature the proteins, so it is necessary to re-extract the proteins for measurement when calculating by protein concentration.

Experimental example:

1. Take 5 μL of rat serum, operate according to the determination steps. Using 96 well plate, calculate ΔA_T = ($A2_T$- $A1_T$) -($A2_B$-$A1_B$)= (0.177-0.068)-(0.080-0.061)=0.090. Bring the result into the standard curve y=0.2439x-0.0119， R^2=0.9999, and calculate x=0.418. The result is calculated according to liquid volume:
   LDL-C content (μmol/dL) =x×100=0.418×100×F=41.8 μmol/dL.
2. Take 5μL of rabbit serum, operate according to the determination steps. Using 96 well plate, calculate ΔA_T = ($A2_T$- $A1_T$) -($A2_B$-$A1_B$)= (0.268-0.067)-(0.080-0.061)=0.182. Bring the result into the standard curve y=0.2439x-0.0119，R^2=0.9999, and calculate x=0.795. The result is calculated according to liquid volume:
   LDL-C content (μmol/dL) =x×100=0.795×100×F=79.5 μmol/dL.
3. Take 0.106g mice liver, add 1 mL of Extract Solution, grind the homogenate with ice bath. Then operate according to the determination steps. Using 96 well plate, calculate ΔA_T = ($A2_T$-$A1_T$)- ($A2_B$-$A1_B$) =(0.107-0.066)-(0.080-0.061)=0.022. Bring the result into the standard curve y=0.2439x-0.0119， R^2=0.9999, and calculate x=0.139. The result is calculated according to sample mass:
   LDL-C content (μmol/g mass) =x÷W =0.139÷0.106×F=1.31 μmol/g mass.