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High-Density Lipoprotein Cholesterol (HDL-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: AK0752-100T-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 I	Self-Provided Reagent	-
Extract Solution II A	Liquid 6 mL×1	2-8℃
Extract Solution II B	Liquid 6 mL×1	2-8℃
Reagent I	Liquid 2 mL×1	2-8℃
Reagent II	Liquid 25 mL×1	2-8℃
Reagent III	Liquid 160 μL×1	2-8℃
Reagent IV	Liquid 25 μL×1	2-8℃
Standard	Powder ×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. **Extract Solution II:** According to the required amount of the experiment, according to the ratio of Extract Solution II A: Extract Solution II B =50 μL: 50 μL (100 μL, 1T), mix well, and prepare before use.
3. **Reagent III:** Shake the liquid from the bottle to the bottom before use. (Use a handheld centrifuge)
4. **Reagent IV:** 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)
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.
6. **Working Solution:** Prepare the working solution according to the ratio of Reagent I: Reagent II: Reagent III: Reagent IV=0.21mL:2.79mL:20**μL**:3**μL** (3.023mL,16T) according to the sample volume, and prepare the working solution as it is used.

Product Description:

High-density lipoprotein is the densest and smallest lipoprotein in serum. Its main function is to transport cholesterol from surrounding tissues to the liver for degradation. Many epidemiological studies have shown that the level of high-density lipoprotein cholesterol is negatively 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 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 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 tissue mass (g): Extract Solution I volume (mL) is 1:5-10. (It is recommended that add 1 mL of Extract Solution I to 0.1 g tissue). Homogenate in ice bath, then centrifuge at 10000 g for 10 minutes at 4℃. Take the supernatant on ice for test.
2. **Bacteria/cells:** according to the number of bacteria/cells (10^6): the volume of Extract Solution I (mL) is 5~10:1. It is recommended that add 1 mL of Extract Solution I 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 for test.
3. **Serum (plasma) or other liquid samples:** detect directly. (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. Standard working solution: Dilute 50 μmol/mL standard solution with **Extract Solution I** to 2.5, 2,1.25, 0.625, 0.3125, 0.15625, 0.078125μmol/mL for standby.
3. Standard dilution table

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

2	50	40	960	2
3	2	625	375	1.25
4	1.25	500	500	0.625
5	0.625	500	500	0.3125
6	0.3125	500	500	0.15625
7	0.15625	500	500	0.078125

Note: The following experiments are requiring 100μL of standard for each standard tube. (Be careful not to test the absorbance of the standard directly in this step.)

4. Operation table: (add Reagent in the 1.5 mL tube)

Reagent (μL)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	100	-	-
Standard	-	100	-
Extract Solution II	100	100	-
Mix well. React at room temperature for 10 minutes. Centrifuge at 12000rpm for 10 minutes at 25℃ and take the supernatant.			
Supernatant	20	20	-
Extract Solution I	-	-	20
Working Solution	180	180	180
Mix well. React at 37℃ for 5min. 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. **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 I (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 HDL-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: HDL-C content (μmol/dL) $=x\times100\times F$
2) Protein concentration: HDL-C content (μmol/mg prot) $=x\times V_E\div(Cpr\times V_E)\times F = x\div Cpr\times F$
3) Sample mass: HDL-C content (μmol/g mass) $=x\times V_E\div W\times F = x\div W\times F$
4) Bacteria/cells number: HDL-C content (μmol/10^6 cell) $=x\times V_E\div N\times F = x\div N\times F$

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

V_E: Added Extract Solution I volume, 1 mL;

W: Sample mass, g;

N: The number of bacteria/cells, $\times 10^6$;

Cpr: The concentration of protein, mg/mL;

F: Sample dilution folds.

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 I (the liquid sample is diluted with distilled water) before the determination. Change the calculation formula simultaneously.
2. Extract solution I contains components that denature proteins, so it is necessary to re-extract proteins for determination when calculating protein concentration.

Experimental example:

1. Take 100μL of rat serum, operate according to the determination steps. Using 96 well plate, calculate $\Delta A_T = A_T - A_B = 0.736 - 0.054 = 0.682$. Bring the result into the standard curve y=0.4526x-0.0077, R^2=0.9992, and calculate x=1.524. The result is calculated according to liquid volume:
   HDL-C content (μmol/dL) =x×100=1.524×100=152.4 μmol/dL.
2. Take 100μL of rabbit serum, operate according to the determination steps. Using 96 well plate, calculate $\Delta A_T = A_T - A_B = 0.985 - 0.054 = 0.931$. Bring the result into the standard curve y=0.4526x-0.0077, R^2=0.9992, and calculate x=2.074. The result is calculated according to liquid volume:
   HDL-C content (μmol/dL) =x×100=2.074×100=207.4 μmol/dL.
3. Take 0.106g mouse liver, add 1 mL of Extract Solution I, grind the homogenate with ice bath. Then operate according to the determination steps. Using 96 well plate, calculate $\Delta A_T = A_T - A_B = 0.207 - 0.054 = 0.153$. Bring the result into the standard curve y=0.4526x-0.0077, R^2=0.9992, and calculate x=0.355. The result is calculated according to sample mass:
   HDL-C content (μmol/g mass) =x÷W =0.355÷0.106=3.349 μmol/g mass.