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

Note: The reagents have been changed, please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer

Catalog Number: BC5330

Size: 50T/48S

Components:

Extract: Isopropyl alcohol 60 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 IA: Liquid 60 mL×1. Store at 2-8℃.

Reagent IB: Liquid 500 μL×1. Store at 2-8℃.

Reagent IC: Liquid 75 μL×1. Store at 2-8℃. The liquid is placed in an EP tube inside a reagent bottle.

Reagent I: According to the ratio of Reagent IA: Reagent IB: Reagent IC=2.25 mL: 20 μL: 3 μL (2.273mL about 3T) to prepare when the solution will be used.

Reagent II: Liquid 20 mL×1. Store at 2-8℃.

Standard Solution: Powder ×1, 10 mg cholesterol. Store at 2-8℃. Add 517 μL of Extract 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 lipoproteins (LDL) are the major carriers of cholesterol in humans, responsible for supplying cholesterol to tissues with the highest sterol demands. Low-density lipoprotein cholesterol (LDL-C) concentrations positively correlate with the incidence of coronary heart disease and a reduction of LDL-C decreases the risk of coronary. Therefore, accurate and precise measurements of patients' LDL-C concentrations are necessary to appropriately identify individuals with atherosclerosis, coronary heart disease and hypertension.

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

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cholesterol content.

Reagents and Equipment Required but Not Provided.

Spectrophotometer, balance, low temperature table centrifuge, constant temperature incubator/water bath, 1mL glass cuvette, pipette, mortar/homogenizer/cell ultrasonic crusher, ice, distilled water, **isopropyl alcohol**.

Procedure

I. Sample preparation:

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. Bacteria/cells: according to the number of bacteria/cells (10^4): the volume of Extract (mL) is 500~1000:1. It suggests that add 1 mL of Extract 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: Directly measured. Centrifuge before detecting if there are precipitation in the liquid.

II. Determination Procedure

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 546 nm and set 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** to 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078125, 0.0390625 μmol/mL for standby.
4. Operation table:

Reagent (μL)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	20	-	-
Standard	-	20	-
Extract	-	-	20
Reagent I	750	750	750
Mix well. React at 37℃ for 5 minutes. Measure the absorption at 546 nm and record as $A1_T$, $A1_S$, $A1_B$.			
Reagent II	250	250	250
Mix thoroughly. React at 37℃ for 5 minutes. Measure the absorption at 546 nm and record as $A2_T$, $A2_S$, $A2_B$. Calculate $\Delta A_T = (A2_T - A1_T) - (A2_B - A1_B)$, $\Delta A_S = (A2_S - A1_S) - (A2_B - A1_B)$. 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 extraction 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 LDL-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: LDL-C content (μmol/dL) =x×100
2) Protein concentration: LDL-C content (μmol/mg prot) =$x \times V_S \div (Cpr \times V_S)$ =x÷Cpr
3) Sample weight: LDL-C content (μmol/g weight) =$x \times Vs \div (W \div V_E \times Vs)$ =x÷W
4) Bacteria/cells number: LDL-C content (nmol/10^4 cell) =$x \times Vs \div (N \div V_E \times Vs) \times 10^3$= x÷N

100: Unit conversion factor, 1 dL=100 mL;
V_S: Added sample volume, 0.02 mL;
V_E: Extract volume, 1 mL;
W: Sample weight, g;
N: The number of bacteria/cells, In tens of thousands;
Cpr: The concentration of protein, mg/mL;
10^3: Unit conversion factor, 1 μmol=10^3 nmol.

Note:

1. If samples ΔA_T is too high, it is suggested that the samples should be diluted with multiple times of Extract solution. Sample supernatant volume could be increased if samples ΔA_T is too low. And modify the calculation formula.
2. The extract contains components that denature the proteins, so it is necessary to reextract the proteins for measurement when calculating by protein concentration.

Experimental example:

1. Take 20 μL of human serum, operate according to the determination steps, calculate ΔA_T = $(A2_T - A1_T) - (A2_B - A1_B)$ = （0.728-0.028）-（0.014-0.013）=0.699. Bring the result into the standard curve y=0.1723x-0.0178 and calculate x=4.160. The result is calculated according to liquid volume:
   LDL-C content (μmol/dL) =x×100=4.160×100=416.019 μmol/dL.
2. Take 0.11g mice liver, add 1 mL of Extract, grind the homogenate with ice bath. Then operate

according to the determination steps, calculate $\Delta A_T = (A2_T - A1_T) - (A2_B - A1_B) = (0.264-0.028) - (0.014-0.013) = 0.235$. Bring the result into the standard curve y=0.1723x-0.0178 and calculate x=1.467. The result is calculated according to sample weight:

LDL-C content (μmol/g weight) =x÷W =1.467÷0.11=13.338 μmol/g weight.

References:

[1] Hiroyuki S, Tetsumi I, Yoshinori U, et al. Homogeneous assay for measuring low-density lipoprotein cholesterol in serum with triblock copolymer and α-cyclodextrin sulfate[J]. Clinical Chemistry, 1998, 44(3):522-531.

[2] Sakaue T, Hirano T, Yoshino G, et al. Reactions of direct LDL-cholesterol assays with pure LDL fraction and IDL: comparison of three homogeneous methods[J]. Clinica Chimica Acta, 2000, 295(1-2):97-106.

Related products:

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BC0750/BC0755	Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit
BC1890/BC1895	Free Cholestenone(FC) Content Assay Kit
BC1980/BC1985	Total Cholestenone(TC) Content Assay Kit
BC5320/BC5325	High-Density Lipoprotein Cholesterol (HDL-C) Content Assay Kit

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