SunLong Biotech Co.,LTD
Tel: 0086-571- 56623320 Fax:0086-571- 56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Low-Density Lipoprotein Cholesterol (LDL-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: AK0692-100T/96S

Size: 100T/96S

Components:

Extract: Isopropyl alcohol 110 mL ×1. Required but not provided. Store at 2-8℃.

Reagent IA: Liquid 25 mL× 1. Store at 2-8℃.

Reagent IB: Liquid 200 μL× 1. Store at 2-8℃.

Reagent IC: Liquid 30 μL× 1. Store at 2-8℃.

Reagent I: According to the ratio of Reagent IA: Reagent IB: Reagent IC=2.25 mL: 20 μL: 3 μL (about 12T) to prepare when the solution will be used.

Reagent ⅡA: Powder ×2. Store at 2-8℃.

Reagent ⅡB: Liquid 10 mL× 1. Store at 2-8℃. Add 4mL of Reagent ⅡB to one Reagent ⅡA and shake to dissolve. It could be stored at 2-8℃ for one week.

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 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 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 is suggest that add 1 mL of Extract 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, 0.078125, 0.0390625 μmol/mL for standby.

3. Operation table:

Reagent (μL)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	5	-	-
Standard	-	5	-
Extract	-	-	5
Reagent I	180	180	180
Mix well. React at 37℃ for 5 minutes. Measure the absorption at 546 nm and record as $A1_T$, $A1_S$, $A1_B$.			
Reagent Ⅱ	60	60	60
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.			

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×V_S÷(Cpr×V_S) =x÷Cpr
3) Sample weight: LDL-C content (μmol/g weight) =x×Vs÷(W÷V_E×Vs) =x÷W
4) Bacteria/cells number: LDL-C content (nmol/10^4 cell) =x×Vs÷(500÷V_E×Vs) ×10^3=2x

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

V_S: Added sample volume, 0.005 mL;

V_E: Extract volume, 1 mL;

W: Sample weight, g;

500: The number of bacteria/cells, 500 million;

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 protein concentration can be detected in another tissue.

Experimental example:

1. Take 5 μL of human serum, operate according to the determination steps, calculate ΔA_T = ($A2_T$ - $A1_T$)- ($A2_B$ - $A1_B$) = (0.515-0.061) - (0.056-0.055) =0.453. Bring the result into the standard curve y=0.1163x-0.0039 and calculate x=3.929. The result is calculated according to liquid volume: LDL-C content (μmol/dL) =x×100=3.929×100=392.863 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 ΔA_T = ($A2_T$ - $A1_T$)- ($A2_B$ - $A1_B$) = (0.239-0.056) - (0.056-0.055) =0.182. Bring the result into the standard curve y=0.1163x-0.0039 and calculate x=1.598. The result is calculated according to sample weight:
   LDL-C content (μmol/g weight) =x÷W =1.598÷0.11=14.531 μmol/g weight.