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Low - Density Lipoprotein Cholesterol (LDL-C) Content

BC5330 (100 Tests)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, low density lipoprotein cholesterol (LDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong positive relationship between LDL-C concentration and the incidence of CHD was reported. LDL Cholesterol acts as a key factor in the pathogenesis of atherosclerosis and coronary artery disease.

This assay method uses a surfactant for selectively solubilizing LDL alone in the cholesterol assay system that employs cholesterol esterase and cholesterol oxidase. It passes the ester cholesterol and free cholesterol contained in the LDL to the cholesterol reaction system to determine LDL cholesterol. The enzyme reactions to other, non-LDL lipoproteins (HDL, VLDL, chylomicrons) are inhibited by the surfactant and by the sugar compounds. These lipoproteins are therefore not passed to the cholesterol reaction system and consequently remain in the reaction liquid as lipoproteins. Based on this principle it is thus possible to directly determine LDL-cholesterol on its own.

Wavelength of absorbance is 600nm and 700nm

Kit components

Reagent	Volume	Storage
Extraction Reagent	1×60mL	2-8°C
LDL –C Direct R1	2 × 30mL	2-8°C
LDL –C Direct R2	2 × 10mL	2-8°C
LDL –C Direct Calibrator	1 × 3mL	2-8°C

Open Vial Stability

Once opened, the reagent is stable up to four weeks at 2-8°C, if contamination is avoided.

Reagent Deterioration

Turbidity or precipitation on in any kit component indicates deterioration and the component must be discarded. Values outside the recommended acceptable range for the Qualicheck Norm & Path control may also be an indication of reagent instability and associated results are invalid. Sample should be retested using a fresh vial of reagent.

Reagent Preparation

Reagent 1 & 2 are ready to use.

Calibrator: Reconstitute with 3mL of distilled water. Let it stand for 2 hours at room temperature. Dissolve the content of the vial by swirling gently to avoid the formation of foam.

Precaution

- To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.
- Avoid direct exposure of reagent to light. Do not blow into the reagent bottles.

Operation Procedures

Sample Preparation

1. Bacteria or cells

Harvest the cells and wash twice with PBS. Ideal to use 5 million cells for the assay. Add 1 mL Extraction Reagent to 5 million cells and ultrasonicate (200W, work time 3 second / interval 10 second repeat for 30 times) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant. The supernatant should be kept on ice.

Note: Ideal proportion of Cells/Bacteria to Extraction Reagent is 1:5-10.

2. Tissue

Prepare 10% tissue homogenate by adding 1 mL Extraction Reagent to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant.

3. Serum or Plasma

Directly use for the assay.

Interferences

No interference for

Bilirubin up to 20 mg/dL

Ascorbic acid up to 50 mg/dL

Haemoglobin up to 500 mg/dL

Triglyceride up to 3000 mg/dL

Materials Required but Not Provided

Pipettes & Tips, Test Tubes & racks, Timer, Incubator, Analyzer

Unit Conversion

Traditional Unit	SI Unit	Conversion from Traditional to SI
mg/dL	mmol/L	x 0.026

Procedure Notes

Reagent	Blank (B)	Calibrator (C)	Sample (S)
Reagent 1	450µL	450µL	450µL
Calibrator	-	5µL	-
Sample	-	-	5µL
Mix & incubate for 5 minutes at 37°C			
Reagent 2	150µL	150µL	150µL
Mix and incubate for 5 minutes at 37°C. Measure the absorbance (OD ₂) at 578nm/630 nm.			

Calculation

ΔA_S = Absorbance of Sample

ΔA_C = Absorbance of Calibrator

LDL-C Concentration (mg/dL) = $A_S - A_C \times 75$

Calibrator concentration: 75 mg/dL

Performance

Linearity

This reagent is linear upto 700 mg/dL

If the concentration is greater than linearity (700 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

Sensitivity

Lower detection Limit is 1.0 mg/dL.