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Ver.250401

High-Density Lipoprotein Cholesterol (HDL-C) Content

BC5320(50 Tests/48 Samples)

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, high density lipoprotein cholesterol (HDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong negative relationship between HDL-C concentration and the incidence of CHD was reported.

The reaction between cholesterol other than HDL and the enzyme for cholesterol assay is suppressed by the electrostatic interaction between polyanions & cationic substances. Hydrogen peroxide is formed by the free cholesterol in HDL by cholesterol oxidase. Oxidative condensation of EMSE and 4-AA is caused by hydrogen peroxide in the presence of peroxidase, and the absorbance of the resulting red-purple Quinone is measured to obtain the cholesterol value in HDL.

Other lipoproteins than HDL $\xrightarrow[\text{Cationic substances}]{\text{Polyanions}}$ Suppress reaction with enzyme

HDL (cholesterol esters) + H₂O $\xrightarrow{\text{Cholesterol esterase}}$ HDL (free cholesterol) + Free fatty acids

HDL (free cholesterol) + O₂ + H⁺ $\xrightarrow{\text{Cholesterol oxidase}}$ Cholestenone + H₂O₂

2H₂O₂ + 4-AA + EMSE + H₃ + O $\xrightarrow{\text{Peroxidase}}$ Violet quinone + 5 H₂O

Wavelength of absorbance is 600 nm and 700 nm

Kit components

Reagent	Volume	Storage
Extraction Reagent	1×60 mL	2-8 ⁰ C
HDL-C Direct R1	2 x 45 mL	2-8 ⁰ C
HDL-C Direct R2	1 x 30 mL	2-8 ⁰ C
HDL-C Direct Calibrator	1 x 3 mL	2-8 ⁰ C

Open Vial Stability

Once opened, the reagent is stable up to four weeks at 2-8⁰C, if contamination is avoided.

Reagent Deterioration

Turbidity or precipitation 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.

Reagent Preparation

The Reagent 1 & Reagent 2 are ready to use.

Calibrator: Reconstitute with 3mL of distilled water. Let it stand for 30 minutes 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.
- This reagent is only for IVD use and follow the normal precautions required for handling all laboratory reagents.

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 1mL Extraction Reagent to 5 million cells and ultrasonicate (200W, work time 3second / 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 1mL 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 40 mg/dL

Ascorbic acid up to 50 mg/dL

Haemoglobin up to 500 mg/dL

Triglyceride up to 1000 mg/dL

(When triglyceride in a sample exceeds 1000 mg/dL, dilute the sample 1+9 with saline, repeat the assay and multiply result by 10)

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

Laboratory procedure for Semi Auto Analyzer			
Reagent	Blank	Calibrator	Sample/control
Reagent 1	270µL	270µL	270µL
Calibrator	-	3µL	-
Sample/Control	-	-	3µL
Mix & incubate for 5 minutes at 37°C.			
Reagent 2	90µL	90µL	90µL
Mix and incubate for 5 minutes at 37°C and read absorbance of calibrator & sample against reagent blank at 600 & 700 nm.			

Calculation

$$\text{HDL-C Concentration (mg/dL)} = \frac{(\text{OD2-OD1}) \text{ Sample}}{(\text{OD2-OD1}) \text{ Calibrator}} \times \text{Calibrator Concentration}$$

Laboratory procedure for Semi Auto Analyzer

Reagent	Blank	Calibrator	Sample/control
Reagent 1	450 µL	450 µL	450 µL
Calibrator	-	5 µL	-
Sample/Control	-	-	5 µL

Mix & incubate for 5 minutes at 37°C.

Reagent 2	150 µL	150 µL	150 µL
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Mix and incubate for 5 minutes at 37°C and read absorbance of calibrator & sample against reagent blank at 578 & 630 nm.

Calculation

$$\text{HDL-C Concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Concentration}$$

Performance

Linearity

This reagent is linear up to 150 mg/dL

If the concentration is greater than linearity (150 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

Sensitivity

Lower detection Limit is 1 mg/dL.