Ver.250401

# High-Density Lipoprotein Cholesterol (HDL-C) Content

BC5320 (50Tests/48Samples)

**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.

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

$$\text{HDL (cholesterol esters)} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{HDL (free cholesterol)} + \text{Free fatty acids}$$

$$\text{HDL (free cholesterol)} + O_2 + H^+ \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{EMSE} + H_3 + O \xrightarrow{\text{Peroxidase}} \text{Violet quinone} + 5\ H_2O$$

Wavelength of absorbance is 600nm and 700nm.

## Kit components

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

## Open Vial Stability

Once opened, the reagent is stable up to four weeks at 2-8℃, 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.

## Reagent Preparation

The Reagent 1 & Reagent 2 are ready to use.

Calibrator: Reconstitute with 3mL of distilled water**.** Let it stand for 30minutes 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 touse5millioncellsfortheassay.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℃ 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℃ 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 |

# 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℃ | | | |
| Reagent 2 | 150µL | 150µL | 150µL |
| Mix and incubate for 5 minutes at 37℃.Measure the absorbance ($OD_2$) at 578nm/630 nm. | | | |

## Calculation

$\Delta A_S$ = Absorbance of Sample

$\Delta A_C$ =Absorbance of Calibrator

HDL-C Concentration (mg/dL) = $A_S$- $A_C$ × 64

Calibrator concentration: 64 mg/dL

## 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.