# High-Density Lipoprotein Cholesterol (HDL-C) Assay Kit

**Catalog No.:** abx294129

**Size:** 100T

**Detection Range:** 0.065 mmol/L – 3.8 mmol/L

**Sensitivity:** 0.065 mmol/L

**Storage:** Store all components at 4°C in the dark.

**Application:** For detection and quantification of HDL-C concentration in serum, plasma, cell lysates, cell culture supernatants, and tissue homogenates.



## Introduction

High-Density Lipoprotein (HDL) particles help clear fats and cholesterol from cells, including atheroma within arterial walls, and transport them to the liver for disposal or reuse. Because of this role, the cholesterol contained in HDL particles (HDL-C) is often referred to as "good cholesterol." Higher HDL-C levels are generally associated with a lower risk of cardiovascular disease, whereas low HDL-C levels are linked to an increased risk of heart disease.

Abbexa's High-Density Lipoprotein Cholesterol (HDL-C) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating HDL-C concentration. Chylomicrons (CM), very-low-density lipoproteins (VLDL) and low density lipoproteins (LDL) coagulate in a polyanionic environment to form a complex. HDL forms a soluble compound under the action of surfactants. The HDL is then able to directly react with reagents containing cholesterol esterase (CE) and cholesterol oxidase (CO) which catalyze HDL to produce hydrogen peroxide. The hydrogen peroxide can then be catalyzed by peroxidase (POD) in the presence of 4-aminoantipyrine (4-AA) and phenol (T-OOS) to form a red quinone compound, with an absorbance maximum at 550 nm. The intensity of the color is proportional to the HDL-C concentration, which can then be calculated.

## Kit components

1. Enzyme Working Solution 1: 75 ml
2. Enzyme Working Solution 2: 25 ml
3. Standard Solution: 1 vial

## Materials required but not provided

1. Spectrophotometer (550 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Normal saline (0.9 % NaCl)
5. Absolute ethanol
6. Pipette and pipette tips
7. 1.5 ml microcentrifuge tubes
8. Centrifuge
9. Vortex mixer
10. Incubator

## Protocol

### A. Preparation of samples and reagents

#### 1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Cell Culture Supernatants:** Centrifuge the supernatant 1000 × g for 10 minutes. Take the supernatant and assay immediately.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue sample, add into 180 µl PBS (0.01 M, pH 7.4) or normal saline (0.9 % NaCl). *If the sample contains a high amount of fat: use absolute ethanol as the homogenization medium*. Homogenize manually, using a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully collect the supernatant. Keep on ice and assay immediately.
- **Cell Lysates:** Collect at least 1 × $10^6$ cells and wash with PBS (0.01 M, pH 7.4). Per 1 × $10^6$ cells, add into 300 µl - 500 µl PBS (0.01 M, pH 7.4) and homogenize manually by ultrasonication at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully collect the supernatant. Keep on ice and assay immediately.

  **Note:** To calculate HDL-C concentration in tissue homogenates or cell lysates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193**).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with PBS (0.01 M, pH 7.4) or normal saline (0.9 % NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

| Sample Type | Dilution Factor |
|---|---|
| Human Serum | 1 |
| Human Plasma | 1 |
| Mouse Serum | 1 |
| Mouse Plasma | 1 |
| Rat Serum | 1 |
| Rat Plasma | 1 |
| Porcine Serum | 1 |

**Note:**

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

## 2. Reagents

- Allow all reagents to equilibrate to room temperature before use.
- Ensure that reagents are protected from contamination with glucose, cholesterol, and other sugars and fats.

## B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark microcentrifuge tubes for each sample, standard and blank. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 10 µl of sample to each sample tube.
3. Add 10 µl of standard to the standard tube.
4. Add 10 µl of double-distilled water to the blank tube.
5. Add 750 µl of Enzyme Working Solution 1 to all tubes.
6. Mix thoroughly and incubate the tubes at 37°C for 5 minutes.
7. Set the spectrophotometer to zero using double-distilled water (0.5 cm optical path cuvette). Measure the OD values of each tube at 550 nm. Record these values as $A_1$.
8. Add 250 µl of Enzyme Working Solution 2 to all tubes.
9. Mix thoroughly and incubate the tubes at 37°C for 5 minutes.
10. Set the spectrophotometer to zero using double-distilled water (0.5 cm optical path cuvette). Measure the OD values of each tube at 550 nm. Record these values as $A_2$.



## C. Calculation of Results

### 1. Serum, Plasma, and Cell Culture Supernatant samples:

$$\text{HDL-C Concetration (mmol/L)} = F \times \frac{(A_{2\ \text{Sample}} - A_{1\ \text{Sample}}) - (A_{2\ \text{Blank}} - A_{1\ \text{Blank}}) \times C_{\text{Standard}}}{(A_{2\ \text{Standard}} - A_{1\ \text{Standard}}) - (A_{2\ \text{Blank}} - A_{1\ \text{Blank}})}$$

**2. Tissue Homogenates and Cell Lysates:**

If PBS (0.01 M, pH 7.4) or normal saline (0.9 % NaCl) used as homogenization medium:

$$\text{HDL-C Concetration (mmol/g protein)} = F \times \frac{(A_{2\ \text{Sample}} - A_{1\ \text{Sample}}) - (A_{2\ \text{Blank}} - A_{1\ \text{Blank}}) \times C_{\text{Standard}}}{(A_{2\ \text{Standard}} - A_{1\ \text{Standard}}) - (A_{2\ \text{Blank}} - A_{1\ \text{Blank}}) \times C_{\text{Protein}}}$$

If absolute ethanol used as homogenization medium (high-fat tissue samples):

$$\text{HDL-C Concetration (mmol/g tissue)} = F \times \frac{(A_{2\ \text{Sample}} - A_{1\ \text{Sample}}) - (A_{2\ \text{Blank}} - A_{1\ \text{Blank}}) \times C_{\text{Standard}} \times V}{(A_{2\ \text{Standard}} - A_{1\ \text{Standard}}) - (A_{2\ \text{Blank}} - A_{1\ \text{Blank}}) \times W}$$

where:

| | |
|---|---|
| $A_{1\ \text{Sample}}$ | OD value of sample after first enzyme reaction |
| $A_{2\ \text{Sample}}$ | OD value of sample after second enzyme reaction |
| $A_{1\ \text{Blank}}$ | OD value of blank after first enzyme reaction |
| $A_{2\ \text{Blank}}$ | OD value of blank after second enzyme reaction |
| $A_{1\ \text{Standard}}$ | OD value of standard after first enzyme reaction |
| $A_{2\ \text{Standard}}$ | OD value of standard after second enzyme reaction |
| $V$ | Volume of absolute ethanol used as homogenization medium (L) |
| $C_{\text{Standard}}$ | Concentration of standard |
| $C_{\text{Protein}}$ | Concentration of protein in sample (g Protein/L) |
| $W$ | The weight of the tissue sample (g) |
| $F$ | The dilution factor of sample |



## Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.