ABS_Bio™ HDL LDL/VLDL Cholesterol Detection Kit  
(Cat# K127-100; 100 assays; store kit at -20°C)

Introduction

Lipoproteins exist in different forms within the blood based on their density. These include chylomicrons, very-low density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), low density lipoproteins (LDLs), and high-density lipoproteins (HDLs). HDL and LDL cholesterol levels in the blood are important indicators of many disease states. Because elevated levels of serum cholesterol is implicated in atherosclerosis and other coronary diseases, cholesterol measurement is of great importance in clinical testing.

The ABS_Bio™ HDL LDL/VLDL Cholesterol Detection Kit provides a simple, sensitive, one-step high-throughput adaptable colorimetric and fluorimetric assay to detect total cholesterol, HDL and LDL/VLDL cholesterol in samples. In this assay, using a convenient separation reagent, HDLs are separated from precipitated LDLs and VLDLs, then cholesteryl esters are hydrolyzed by cholesteryl esterase into cholesterol, which is then oxidized by cholesterol oxidase to yield the corresponding ketone and H₂O₂. In the presence of Horseradish Peroxidase (HRP), H₂O₂ converts the colorless substrate into a colored product which can be measured by spectrophotometry or fluorometry. The intensity of the color, measured at 570 nm, or the fluorescence at Ex/Em=530/590nm, is directly proportional to the amount of total cholesterol or free cholesterol in the sample. The kit is supplied with sufficient reagents for 100 tests in a 96-well plate assay and has a linear detection range of 1.2-500 µM (0.12-50 μM fluorimeter) cholesterol with only 20 µL sample volume. It could easily be modified for use in a 384-well or a high-throughput assay.

Kit Components (100 tests)

| Assay Buffer: 20 mL | Enzyme Mix: 0.12 mL | Probe: 0.12 mL | Cholesterol Standard (10 mM): 0.5 mL | Enzyme A: 0.12 mL | Precipitation Solution: 5 mL | PBS: 5 mL |

Storage and Handling: Store kit at -20°C. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Add 50 µL of sample (serum or plasma) to a microcentrifuge tube. Add 50 µL of the precipitation solution and mix well by vortexing. Allow the mixture to incubate for 5-10 minutes at room temperature. Centrifuge the mixture at 9000 x g (~9000 rpm) for 5 minutes. Slowly and carefully transfer the supernatant (HDL fraction) into a new tube, leaving the pellet (LDL/VLDL fraction). Resuspend and dissolve the pellet in 100 µL of PBS, vortex well. Ensure that the pellet (LDL/VLDL fraction) is completely dissolved before testing. Further dilute the serum, HDL or LDL/VLDL fraction samples from 1:5 (1:10 final dilution) to 1:20 (1:40 final dilution) in assay buffer to ensure the readings are within the standard curve range. Transfer 20 µL of sample (serum, HDL fraction, LDL/VLDL fraction) into the clear 96-well plate in duplicate.

2. Standard Preparation

Transfer 20 µL of the 10 mM cholesterol standard into 380 µL assay buffer to generate a 500 µM cholesterol standard, then use the following table to generate 500, 300, 150, 50 and 0 µM cholesterol standards.

<table>
<thead>
<tr>
<th>500 µM Cholesterol std(µL)</th>
<th>Assay buffer(µL)</th>
<th>final cholesterol concentration µM (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
<td>500 (19.31)</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>300 (11.58)</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>150 (5.79)</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>50 (1.93)</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

Transfer 20 µL of appropriate standards into the clear 96-well plate in duplicate. The blank control contains buffer only.

3. Reaction

For total cholesterol measurement: Prepare enough working reagent by mixing 80 µL assay buffer, 1 µL enzyme A, 1 µL enzyme mix and 1 µL probe for each reaction (samples & standards).

For free cholesterol measurement (Option): Prepare enough working reagent by mixing 81 µL assay buffer, 1 µL enzyme mix and 1 µL probe for each reaction (samples & standards).

The assay is based on an enzyme-catalyzed kinetic reaction, use of a multi-channel pipettor is recommended to transfer 80 µL of prepared working reagent into each reaction well. Tap plate to mix well. Incubate for 40 min. at room temperature, protected from light.

4. Measurement

Read the optical density at 570 nm (550-590 nm).

5. Calculation

Average the duplicate OD570 nm reading for the standard and the sample. Subtract the average OD of the blank from the average OD of the standards and plot the result (ΔOD) versus the cholesterol concentration of the standards. Determine the slope by linear regression and calculate the cholesterol concentration of the samples.

\[ [\text{Cholesterol}] = \frac{(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}})}{\text{Slope} \times n (\muM)} \]

\( n \) is the sample dilution factor.

Cholesteryl Ester (µM) = Total Cholesterol - Free Cholesterol

\( \text{OD}_{\text{sample}} \) and \( \text{OD}_{\text{blank}} \) are the optical density of the sample and buffer.

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NOTE: To convert the results from µM to mg/dL, divide the cholesterol concentration (µM) by 25.9.

Typical Standard Curve

Cholesterol standard in 96 wells-plate assay (colorimetric). Serum sample in 96 wells-plate assay (colorimetric).

Always run your own standard curves for calculation of results.

**Fluorimetric assay has around a 10-fold sensitivity of the colorimetric assay.**

The prepared cholesterol standard from above should be diluted 10-fold more with assay buffer to generate 50, 30, 15, 5 and 0 µM cholesterol standards.

Transfer 20 µL of the appropriate standards and samples into a 96-well black microplate in duplicate.

For total cholesterol measurement: Prepare enough working reagent by mixing 80 µL assay buffer, 1 µL enzyme A, 1 µL enzyme mix and 0.5 µL probe for each reaction (samples & standards).

For free cholesterol measurement (option): Prepare enough working reagent by mixing 81 µL assay buffer, 1 µL enzyme mix and 0.5 µL probe for each reaction (samples & standards).

Transfer 80 µL of prepared working reagent into each reaction well. Tap plate to mix well. Incubate for 40 min. at room temperature, protected from light.

Read fluorescence at Ex=530nm and Em=590nm. The cholesterol concentration of sample is calculated as:

\[
[\text{Cholesterol}] = \frac{(\text{RFU}_{\text{sample}} - \text{RFU}_{\text{blank}}) \times \text{n}}{\text{Slope}} \times (\mu M)
\]

RFU\text{sample} and RFU\text{blank} are the related fluorescence units of the sample and assay buffer. n is the sample dilution factor.

**Sensitivity and Limit of Detection**

The Limit of Detection was determined at 1.2 µM, with a linear detection range up to 500 µM in a 96-well plate colorimetric assay, and 0.12-50 µM in a 96-well plate fluorimetric assay. Sensitivity was determined at 0.1 µM. Samples with values above 500 µM should be dilute with assay buffer, re-assayed, and multiplied by the dilution factor.

**Interferences**

Culture media containing phenol red in DMEM (15mg/L) and RPMI 1640 (5mg/L) were tested in the assay for interference in assay buffer. No significant change in the measured cholesterol level was observed.

**References**


**Related Products:**

- Uric Acid Detection Kit (#K152-100)
- Creatinine Detection Kit (#K148-250)
- Glucose Detection Kit (#K188-100)
- Glutathione Detection Kit (#K140-100)
- Peroxidase Activity Detection Kit (#K126-200)
- L-Lactate Fluorimetric Detection Kit (#K208-100)
- L-Lactate Colorimetric Detection Kit (#K207-100)
- Cholesterol Detection Kit (#K128-100)
- Cholesterol Colorimetric Detection Kit (#K328-100)
- NAD/NADH Detection Kit (#K119-100)
- Nitric Oxide Detection Kit (#K116-100)
- Phosphatase Detection Kit (#K196-500)
- Urea Detection Kit (#K158-200)
- Glutathione Peroxidase Detection Kit (#K143-100)
- Glutathione Reductase Detection Kit (#K146-100)
- Hemoglobin Detection Kit (#K168-200)
- Phosphate Detection Kit (#K198-500)
- HDL LDL/VLDL Cholesterol Colorimetric Detection Kit (K327-100)