High-density Lipoprotein Cholesterol (HDL-C) Assay Kit
(Double reagents)

**Catalog No:** E-BC-K221  
**Method:** Colorimetric method  
**Specification:** 96T

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)    Fax: 240-252-7376(USA)  
Email: techsupport@elabscience.com  
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.
Application
This kit can be used for detection of high-density lipoprotein cholesterol (HDL-C) content in serum, plasma, cells, culture supernatant and tissue samples.

Detection principle

\[
\text{HDL} + \text{VLDL} + \text{CM} \xrightarrow{\text{Polymeric compound}} \text{Complex}
\]

\[
\text{HDL} \xrightarrow{\text{Surfactant}} \text{HDL}
\]

\[
\text{HDL-cholesterol} \xrightarrow{\text{CO,CE}} \Delta^4\text{-Cholesterol} + H_2O
\]

\[
H_2O_2 + 4\text{-AAP + Toos} \xrightarrow{\text{POD}} \text{Red purple pigment}
\]

The generated red purple pigment have a maximum absorption peak at 546 nm. Measure the OD value at 546 nm and the HDL-C content in the sample can be calculated.

Kits components

<table>
<thead>
<tr>
<th>Reagent composition</th>
<th>Specification</th>
<th>Component</th>
<th>Concentration</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>18 mL × 1 vial</td>
<td>Good’s Buffer</td>
<td>50 mmol/L</td>
<td>2~8°C Shading light</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Toos</td>
<td>1 mmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MgCl₂·6H₂O</td>
<td>15 mmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cholesterol oxidase</td>
<td>≥ 3 KU</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peroxidase (POD)</td>
<td>≥ 5 KU</td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>6 mL × 1 vial</td>
<td>Good’s Buffer</td>
<td>50 mmol/L</td>
<td>2~8°C Shading light</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-ampyrene</td>
<td>0.2 mmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MgCl₂·6H₂O</td>
<td>15 mmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cholesterol esterase</td>
<td>≥ 3 KU</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surfactant</td>
<td>0.1%</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>Powder × 1 vial</td>
<td>Cholesterol</td>
<td>1.10 mmol/L</td>
<td></td>
</tr>
</tbody>
</table>

The preparation of the standard: dissolve a vial of standard powder with 200 μL double distilled water before use.

Experimental instrument
Test tube, Micropipettor, Vortex mixer, Water bath, Microplate reader or Biochemical analyzer (546 nm)
Sample treatment

1. **Serum (Plasma):** Detect the sample directly. If the concentration is beyond the linear range, then dilute the sample with normal saline before detection.

2. **Culture supernatant sample:** Collect the culture medium, centrifuge at 1000 rpm for 10 min, and take the supernatant for detection.

   [Note]: It is generally recommended that the cell density should be more than 1×10⁶/mL.

3. **Tissue sample:** Accurately weigh the tissue weight, add 9 times the volume of homogenate media according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 2500 rpm for 10 min, then take the supernatant for detection.

   [Note]: (1) If the tissue sample is not a high-fat sample, the homogenate media should be phosphate buffer (0.1 mol/L, pH 7.4) or normal saline.

   (2) If the tissue sample is high-fat sample or partly high lipid sample, the homogenate media should be absolute alcohol.

4. **Cell sample:**

   - **Cell collection:** Take the prepared cell suspension and centrifuge at 1000 rpm for 10 min. Discard the supernatant and keep the cell sediment. Wash the sediment with iso-osmia buffer (0.1 mol/L, pH7~7.4 phosphate buffer was recommended) 1~2 times, centrifuge at 1000 rpm for 10 min. Discard the supernatant and keep the cell sediment.

   - **Cell disruption:** Add 0.2~0.3 mL of homogenate media (0.1 mol/L, pH7~7.4 phosphate buffer or normal saline was recommended). Sonicate in ice water bath (power: 300 W, 3~5 second/time, interval for 30 sec, repeat for 3~5 times) or grind with hand-operated. The prepared homogenate kept for detection without centrifugation. The cell can also be lysed with the cell lysate buffer (Triton X-100, 1~2%, 30~40 min), then take the prepared lysate for detection directly without centrifugation.

   [Note]: It is generally recommended that the cell density should be more than 1×10⁶/ml. The disrupted cell can be observed with microscope that whether the cell is broken completely.

**Operation steps**

<table>
<thead>
<tr>
<th>Operate with 96T microplate. Colorimetric assay by microplate reader</th>
<th>Blank well</th>
<th>Standard well</th>
<th>Sample well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (μL)</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard (μL)</td>
<td></td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Sample (μL)</td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Reagent 1 (μL)</td>
<td>180</td>
<td>180</td>
<td>180</td>
</tr>
</tbody>
</table>

Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A1) of each well at 546 nm with microplate reader.

| Reagent 2 (μL) | 60 | 60 | 60 |

Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A2) of each tube at 546 nm with microplate reader.
Operate with automatic biochemical analyzer

<table>
<thead>
<tr>
<th>The volume of sample/distilled water</th>
<th>2.5 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>180 µL</td>
</tr>
</tbody>
</table>

Mix fully and incubate at 37°C for 5 min. Measure the OD value (A1) of each well at 546 nm with microplate reader.

| Reagent 2                           | 60 µL  |

Mix fully and incubate at 37°C for 5 min. Measure the OD value (A2) of each tube at 546 nm with microplate reader.

Main wavelength | 546 nm |

Reaction type | Endpoint method |

Reaction direction | Up reaction (+)

Calculation of results

1. For serum and other liquid sample:
   Operated with microplate reader:
   \[
   \text{HDL} - C \text{ Content (mmol/L)} = \frac{(A2_{\text{Sample}} - A1_{\text{Sample}}) - (A2_{\text{Blank}} - A1_{\text{Blank}})}{(A2_{\text{Standard}} - A1_{\text{Standard}}) - (A2_{\text{Blank}} - A1_{\text{Blank}})} \times \text{Concentration of standard (mmol/L)}
   \]

   Operated with automatic biochemical analyzer:
   \[
   \text{HDL} - C \text{ Content (mmol/L)} = \frac{(A2_{\text{Sample}} - A1_{\text{Sample}})}{(A2_{\text{Standard}} - A1_{\text{Standard}})} \times \text{Concentration of standard (mmol/L)}
   \]

2. For tissue and cell sample:
   Operated with microplate reader:
   \[
   \text{HDL} - C \text{ Content (mmol/gprot)} = \frac{(A2_{\text{Sample}} - A1_{\text{Sample}}) - (A2_{\text{Blank}} - A1_{\text{Blank}})}{(A2_{\text{Standard}} - A1_{\text{Standard}}) - (A2_{\text{Blank}} - A1_{\text{Blank}})} \times \text{Concentration of standard (mmol/L)} \div \text{Protein concentration of tested sample (gprot/L)}
   \]

   Operated with automatic biochemical analyzer:
   \[
   \text{HDL} - C \text{ Content (mmol/gprot)} = \frac{(A2_{\text{Sample}} - A1_{\text{Sample}})}{(A2_{\text{Standard}} - A1_{\text{Standard}})} \times \text{Concentration of standard (mmol/L)} \div \text{Protein concentration of tested sample (gprot/L)}
   \]
Performance index

1. The absorbance of blank tube is $\leq 0.010$ (optical path = 0.5 cm).
2. **Linear range:** 0.065~3.8 mmol/L, $r^2 > 0.995$.
3. **Sensitivity:** The absorbance value of $\Delta A$ is between 0.087 ~ 0.153 when testing 1.3 mmol/L samples.
4. **Accuracy:** Relative deviation $\leq 10\%$.
5. **Precision:** intra-CV $\leq 3\%$, inter-CV $\leq 5\%$.
6. **Stability:** The validity of kit is 12 months when stored at 2°C~8°C in the dark. It is stable for 1 month when stored at 2°C~8°C in the dark after opening.

Notes

1. This product is for scientific research use only, not for clinical diagnosis.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.
5. If the sample content is beyond the maximum limit, please dilute the sample with normal saline before detection, and multiply the result by the dilution ratio.
6. Protect the reagent from contamination of glucose, cholesterol, etc.
7. The amount of reagent and sample can be increased and decreased proportionately according to the volume of cuvette.