HDL DIRECT
(Direct Method)
Reagent for quantitative estimation of HDL-Cholesterol in Serum or Plasma.

SUMMARY:
High- Density Lipoproteins (HDL) are one of the major classes of plasma lipoproteins. They are composed of a number of heterogeneous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serves to remove cholesterol from the peripheral cells of the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL – Cholesterol (HDL-C) levels in serum and the incidence/prevalence of Coronary Heart Disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognized. An accurate measurement of HDL-C is of vital importance when assessing patient risk from CHD.

PRINCIPLE:
Specific and special detergent react with HDL-C fractions, followed by enzymatic reactions with CE, CO, POD and with Chromogenic coupler leading to form color, simultaneously non HDL-C Lipoproteins (CM,VLDL & LDL) form colorless water-soluble compound with other group of specific detergents. The HDL-C fraction reaction as per below;

\[
\begin{align*}
\text{HDL - Cholesterol Esters} & \xrightarrow{\text{CE}} \text{Cholesterol + fatty acids} \\
\text{Cholesterol + O2} & \xrightarrow{\text{CO}} \text{Cholesterol-4-en-3-One + H2O2} \\
2\text{H2O2} + 4\text{AAP} + \text{Phenolic Compound} & \xrightarrow{\text{POD}} \text{Quinoneimine dye + 4H2O}
\end{align*}
\]

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<th>PACK SIZE</th>
<th>HDL-C REAGENT</th>
<th>HDL-C CALIBRATOR</th>
</tr>
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<tbody>
<tr>
<td>1×32 ml</td>
<td>1×32 ml</td>
<td>1×1 ml</td>
</tr>
</tbody>
</table>

SAMPLE:

On empty stomach Serum and heparinized plasma are acceptable. EDTA plasma is acceptable, but causes decreased results. Do not freeze the samples. If any sample show precipitation, centrifuge before using.

HDL CALIBRATOR PREPARATION & STABILITY:

For reconstitution refer the Calibrator vial label. After reconstitution Calibrator stable for 7 days at 2-8°C.

REAGENT STORAGE & STABILITY:

All Reagents included in the kit are stable at 2-8°C until the expiry date stated on the label.
PREPARATION OF WORKING REAGENT:

D-HDL Cholesterol Reagent Ready To Use.

GENERAL SYSTEM PARAMETER:

<table>
<thead>
<tr>
<th>Reaction Mode</th>
<th>End point</th>
<th>Sample Volume</th>
<th>5 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength1/wavelength2</td>
<td>620 nm/700 nm</td>
<td>Reagent volume</td>
<td>400 µl</td>
</tr>
<tr>
<td>Blank</td>
<td>Reagent</td>
<td>Calibrator Conc.</td>
<td>As on vial</td>
</tr>
<tr>
<td>Incubation</td>
<td>5 mins at 37°C</td>
<td>Reaction Slope</td>
<td>Increasing</td>
</tr>
<tr>
<td>Delay Time</td>
<td>-</td>
<td>Linearity</td>
<td>250 mg/dl</td>
</tr>
<tr>
<td>Read Time</td>
<td>-</td>
<td>Units</td>
<td>mg/dl</td>
</tr>
</tbody>
</table>

ASSAY PROCEDURE:

<table>
<thead>
<tr>
<th>Pipette into Test Tube</th>
<th>Blank</th>
<th>Calibrator</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C Reagent</td>
<td>400 µl</td>
<td>400 µl</td>
<td>400 µl</td>
</tr>
<tr>
<td>HDL-C Calibrator</td>
<td>-</td>
<td>5 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>5 µl</td>
</tr>
</tbody>
</table>

Mix & incubation for 5 mins at 37°C. Read Abs of Test and Calibrator against blank at 620 nm

CALCULATIONS:

HDL-C (mg/dl) = Abs T + Abs C X Conc. of Calibrator

NORMAL REFERENCE VALUES:

Male: 35 - 80 mg/dl
Female: 42 - 88 mg/dl

LINEARITY:

This procedure is linear up to 250 mg/dl.

NOTE:

A special surfactant, Lipid Clearing Factor (L.C.F.) is added to the Reagent to solubilise the lipemic sera (causing turbidity or opalescence) which adds to the accuracy of results

REFERENCES:


For In Vitro Diagnostic Use Only