HDL-c direct FS*

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 3561 99 10 920</td>
<td>800 (4 x 200)</td>
</tr>
<tr>
<td>1 3561 99 10 921</td>
<td>480 (4 x 120)</td>
</tr>
</tbody>
</table>

Intended Use

Diagnostic reagent for quantitative in vitro determination of HDL-C (high density lipoprotein cholesterol) in serum and plasma on respons®910.

Summary

Cholesterol, synthesized by body cells and absorbed with food, is a component of cell membranes and a precursor for steroid hormones and bile acids. Cholesterol is transported in plasma via lipoproteins, complexes between lipids and apolipoproteins. Four lipoprotein classes exist: High density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. These classes show distinct relationship to coronary atherosclerosis. LDL is involved in the cholesterol transport to the peripheral cells, contributing to atherogenesis. HDL cholesterol (HDL-C) has a protective effect impeding plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol (TC) within the normal range, an increased concentration of LDL-cholesterol (LDL-C) indicates high risk. HDL-cholesterol (HDL-C) has a protective effect on plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-C values constitute an independent risk factor. One of the important functions of HDL includes the physiological removal of cholesterol from peripheral tissues and cells, and transport to the liver. The concept that HDL could protect against CHD was primarily originated from epidemiological studies of the healthy population, in particular the Framingham study. In addition to a number of antioxidant effects, HDL also serves as a powerful mediator of the cellular inflammatory and antithrombotic responses. HDL-particles are macromolecule complexes synthesized by liver and intestine and formed from surface components. HDL-particles are released into plasma during lipolysis of lipoproteins rich in triglycerides. Particles consist of an amphipathic lipid monolayer of phospholipids and cholesterol with embedded amphipathic proteins surrounding a core of hydrophobic lipids, mostly cholesteryl esters and triglycerides. HDL-C monitoring is highly relevant in cardiovascular risk assessment. Elevated HDL-C levels usually correlate with decreased cardiovascular risk; whereas reduced concentrations of HDL-C, especially in combination with elevated triglycerides are associated with high risk of atherosclerotic heart disease, even at or below recommended LDL-C goals. Preferred screening tests for dyslipidemia or lipid disorders are TC and HDL-C but the majority of screening guidelines nowadays recommend a full lipid profile including TC, LDL-C, HDL-C and triglycerides. [1-8]

Method

Previous HDL-cholesterol determinations were performed by time-consuming precipitation methods or ultracentrifugation (reference method in combination with cholesterol measurement by Abell-Kendall). However, the direct determination of HDL-cholesterol is used in routine [9]. HDL-c direct FS is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Block polymer detergents protect LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement [10].

HDL-cholesterol ester → CHE & CHO → \( \Delta^4 \)-Cholestenon + free fatty acids + \( \text{H}_2\text{O} \)

\( \text{H}_2\text{O} + 4\text{-Aminoorantipyrine} + \text{H-DAOs} \) → POD → Blue dye + \( \text{H}_2\text{O} \)

The intensity of the formed dye is directly proportional to the cholesterol concentration and is measured photometrically.

Reagents

Components and Concentrations

| R1 | Buffer pH 6.85 | 20 mmol/L |
| N-Peroxidase (POD) | ≥ 2000 U/L |
| N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (H-DAOs) | ≥ 0.7 mmol/L |
| R2 | Buffer pH 8.15 | 20 mmol/L |
| Cholesterol esterase (CHE) | ≥ 400 U/L |
| Cholesterol oxidase (CHO) | ≥ 700 U/L |
| Peroxidase (POD) | ≥ 15000 U/L |
| 4-Aminoorantipyrine | ≥ 1.5 mmol/L |

Storage and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents and protect them from light.

DiaSys respons containers provide protection from light.

Warnings and Precautions


2. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.

3. The reagents contain animal material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.

4. Acetaminophen and metamizole medication leads to falsely low results in patient samples.

5. In very rare cases, samples of patients with gammopathy might give falsified results [11].

6. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.

7. For professional use only.

Waste Management

Refer to local legal requirements.

Reagent Preparation

The reagent is ready to use. The bottles are placed directly into the reagent rotor.

Materials Required

General laboratory equipment

Specimen

Serum and heparin plasma (Lithium)

Stability [12]:

| 2 days | at | 20 – 25°C |
| 7 days | at | 4 – 8°C |
| 3 months | at | –20°C |

Only freeze once. Discard contaminated specimens.

Calibrators and Controls

DiaSys TruCal Lipid is recommended for calibration. TruCal Lipid calibrator values have been made traceable to NIST SRM® 1951 Level 2. Use DiaSys TruLab L for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruCal Lipid</td>
<td>1 3570 99 10 045</td>
</tr>
<tr>
<td>TruLab L Level 1</td>
<td>5 9020 99 10 065</td>
</tr>
<tr>
<td>TruLab L Level 2</td>
<td>5 9030 99 10 065</td>
</tr>
</tbody>
</table>
Performance Characteristics

Exemplary data for serum mentioned below may slightly differ in case of deviating measurement conditions.

<table>
<thead>
<tr>
<th>Interfering substance</th>
<th>Interferences &lt; 10% up to</th>
<th>Analyte concentration [mg/dL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbate</td>
<td></td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 mg/dL 81.0</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td>800 mg/dL 31.2</td>
</tr>
<tr>
<td>Bilirubin (conjugated)</td>
<td></td>
<td>40 mg/dL 38.8</td>
</tr>
<tr>
<td>Bilirubin ( unconjugated)</td>
<td></td>
<td>40 mg/dL 79.4</td>
</tr>
<tr>
<td>Lipemia (triglycerides)</td>
<td></td>
<td>1000 mg/dL 80.7</td>
</tr>
<tr>
<td>N-acetylcysteine (NAC)</td>
<td></td>
<td>1700 mg/L 38.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1700 mg/L 74.3</td>
</tr>
</tbody>
</table>

For further information on interfering substances refer to Young DS [13, 14].

Precision

<table>
<thead>
<tr>
<th>Within run (n=20)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean [mg/dL]</td>
<td>17.9</td>
<td>44.4</td>
<td>183</td>
</tr>
<tr>
<td>CV [%]</td>
<td>2.49</td>
<td>1.58</td>
<td>1.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total precision CLSI (n=80)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean [mg/dL]</td>
<td>18.2</td>
<td>45.2</td>
<td>188</td>
</tr>
<tr>
<td>CV [%]</td>
<td>4.44</td>
<td>2.66</td>
<td>2.82</td>
</tr>
</tbody>
</table>

Method comparison (n=146)

<table>
<thead>
<tr>
<th>Test x</th>
<th>DiaSys HDL-c direct FS BioMajesty®-JCA-BM0010/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test y</td>
<td>DiaSys HDL-c direct FS (respons*910)</td>
</tr>
<tr>
<td>Slope</td>
<td>1.02</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.313 mg/dL</td>
</tr>
<tr>
<td>Coefficient of correlation</td>
<td>0.996</td>
</tr>
</tbody>
</table>

** according to CLSI document EP17-A2; Vol. 32, No. 8

Conversion Factor

HDL-C [mg/dL] x 0.02586 = HDL-C [mmol/L]

Reference Range

As follows [15]:

National Cholesterol Education Program (NCEP) guidelines:

Low HDL-cholesterol (major risk factor for CHD): ≤ 40 mg/dL (≤ 1.04 mmol/L)

High HDL-cholesterol (“negative” risk factor for CHD):

≥ 60 mg/dL (≥ 1.55 mmol/L)

A number of factors contribute to low HDL-cholesterol levels: e.g. overweight and obesity, smoking, physical inactivity, drugs such as beta-blockers and progestational agents, genetic factors.

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

HDL-c direct FS

Application for serum and plasma samples

This application was set up and evaluated by DiaSys. It is based on the standard equipment at that time and does not apply to any equipment modifications undertaken by unqualified personnel.

Identification

- This method is usable for analysis: Yes
- Twin reaction: No
- Name: HDLCD
- Reagent barcode reference: 072

Technic

- Type: End point
- First reagent [µL]: 180
- Blank reagent: Yes
- Sensitive to light: Yes
- Main wavelength [nm]: 600
- Secondary wavelength [nm]: 700
- Polychromatic factor: 1.000
- 1st reading time [min:sec]: 04:24
- Last reading time [min:sec]: 10:00
- Reaction way: Increasing

Linear Kinetics
- Substrate depletion: Absorbance II
- Linearity: Maximum deviation [%]

Fixed Time Kinetics
- Substrate depletion: Absorbance limit
- Endpoint
- Stability: Largest remaining slope
- Prozone Limit [%]

Sample

- Diluent: DIL A (NaCl)
- Hemolysis: 0 (no hemolysis)
- Cleaner: Sample [µL]: 0
- Technical limits
  - Concentration technical limits-Lower: 3
  - Concentration technical limits-Upper: 200
  - SERUM
    - Normal volume [µL]: 3
    - Normal dilution (factor): 1
    - Below normal volume [µL]: 6
    - Below normal dilution (factor): 1
    - Above normal volume [µL]: 3
    - Above normal dilution (factor): 6
  - URINE
    - Normal volume [µL]: 3
    - Normal dilution (factor): 1
    - Below normal volume [µL]: 6
    - Below normal dilution (factor): 1
    - Above normal volume [µL]: 3
    - Above normal dilution (factor): 6
  - PLASMA
    - Normal volume [µL]: 3
    - Normal dilution (factor): 1
    - Below normal volume [µL]: 6
    - Below normal dilution (factor): 1
    - Above normal volume [µL]: 3
    - Above normal dilution (factor): 6
  - CSF
    - Normal volume [µL]: 3
    - Normal dilution (factor): 1
    - Below normal volume [µL]: 6
    - Below normal dilution (factor): 1
    - Above normal volume [µL]: 3
    - Above normal dilution (factor): 6
  - Whole blood
    - Normal volume [µL]: 3
    - Normal dilution (factor): 1
    - Below normal volume [µL]: 6
    - Below normal dilution (factor): 1
    - Above normal volume [µL]: 3
    - Above normal dilution (factor): 6

Results

- Decimals: 2
- Units: mg/dL
- Correlation factor-Offset: 0.000
- Correlation factor-Slope: 1.000

Range

- Gender: All
- Age
- ERUM: >=40 <=200
- PLASMA: >=40 <=200
- CSF: Whole blood
- Whole blood

Contaminants

- Please refer to r910 Carryover Pair Table

Calibrators details

<table>
<thead>
<tr>
<th>Calibrator list</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal. 1/Blank</td>
<td>0</td>
</tr>
<tr>
<td>Cal. 2</td>
<td>0.005</td>
</tr>
<tr>
<td>Cal. 3</td>
<td>0.015</td>
</tr>
<tr>
<td>Cal. 4</td>
<td>0</td>
</tr>
<tr>
<td>Cal. 5</td>
<td>0</td>
</tr>
<tr>
<td>Cal. 6</td>
<td>0</td>
</tr>
</tbody>
</table>

Max delta abs.

<table>
<thead>
<tr>
<th>Cal.</th>
<th>Max delta abs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal. 1</td>
<td>0.8</td>
</tr>
<tr>
<td>Cal. 2</td>
<td>0.8</td>
</tr>
<tr>
<td>Cal. 3</td>
<td>0.8</td>
</tr>
<tr>
<td>Cal. 4</td>
<td>0.8</td>
</tr>
<tr>
<td>Cal. 5</td>
<td>0.8</td>
</tr>
<tr>
<td>Cal. 6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Calculations

<table>
<thead>
<tr>
<th>Model</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>1</td>
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</tbody>
</table>

* Enter calibrator value