Cholesterol FS*

Diagnostic reagent for quantitative in vitro determination of cholesterol in serum or plasma on photometric systems

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
<th>1 x</th>
<th>3 mL Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1300 99 10 021</td>
<td>R</td>
<td>5 x</td>
<td>25 mL</td>
</tr>
<tr>
<td>1300 99 10 026</td>
<td>R</td>
<td>6 x</td>
<td>100 mL</td>
</tr>
<tr>
<td>1300 99 10 023</td>
<td>R</td>
<td>1 x</td>
<td>1000 mL</td>
</tr>
<tr>
<td>1300 99 10 704</td>
<td>R</td>
<td>8 x</td>
<td>50 mL</td>
</tr>
<tr>
<td>1300 99 10 717</td>
<td>R</td>
<td>6 x</td>
<td>100 mL</td>
</tr>
<tr>
<td>1300 99 10 917</td>
<td>R</td>
<td>10 x</td>
<td>60 mL</td>
</tr>
<tr>
<td>1300 99 90 314</td>
<td>R</td>
<td>12 x</td>
<td>25 mL</td>
</tr>
<tr>
<td>1300 99 10 030</td>
<td>R</td>
<td>6 x</td>
<td>3 mL</td>
</tr>
</tbody>
</table>

Summary [1,2]

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food. Cholesterol is transported in plasma via lipoproteins, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins: high density lipoproteins (HDL), very low density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. LDL-cholesterol (LDL-C) contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol within the normal range an increased concentration of LDL-C indicates high risk. HDL-C has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-C values constitute an independent risk factor. The determination of the individual total cholesterol (TC) level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL-C and LDL-C.

In the last few years several controlled clinical trials using diet, life style changes and/or different drugs (especially HMG CoA reductase inhibitors [statins]) have demonstrated that lowering total cholesterol within the normal range an increased concentration of LDL-C contributes to atherosclerotic plaque formation.

Waste Management

The reagent and the standard are ready to use. Reagent and standard are stable up to the end of the indicated month of expiry, if stored at 2–8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Material disposition

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


3. In very rare cases, samples of patients with gammapathy might give falsified results [8].

4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.

Storage Instructions and Reagent Stability

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


Materials required but not provided

NaCl solution 9 g/L

General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma

Stability [6]:

- 7 days at 20 – 25°C
- 7 days at 4 – 8°C
- 3 months at −20°C

Discard contaminated specimens! Freeze only once!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength 500 nm, Hg 546 nm

Optical path 1 cm

Temperature 20 – 25°C/37°C

Measurement Against reagent blank

Sample or standard Blank Sample or standard

<table>
<thead>
<tr>
<th>Sample or standard Blank</th>
<th>10 µL</th>
<th>Sample or standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dist. water</td>
<td>10 µL</td>
<td></td>
</tr>
<tr>
<td>Reagent</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
</tbody>
</table>

Mix, incubate for 20 min. at 20 – 25°C or for 10 min. at 37°C. Read absorbance within 60 min against reagent blank.

Method

"CHOD-PAP": enzymatic photometric test

**Principle**

Determination of cholesterol after enzymatic hydrolysis and oxidation [3,4]. The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder’s reaction) [3].

\[
\text{Cholesterol ester + H}_2\text{O} \rightarrow \text{CHE} \rightarrow \text{Cholesterol + Fatty acid} \\
\text{Cholesterol + O}_2 \rightarrow \text{CHO} \rightarrow \text{Cholesterol-3-one + H}_2\text{O}_2 \\
\text{2 H}_2\text{O}_2 + 4\text{-Aminoantipyrine + Phenol} \rightarrow \text{POD} \rightarrow \text{Quinoneimine + 4 H}_2\text{O} \\
\]

Reagents

Components and Concentrations

<table>
<thead>
<tr>
<th>Reagent</th>
<th>pH</th>
<th>50 mmol/L</th>
<th>5 mmol/L</th>
<th>0.3 mmol/L</th>
<th>≥ 200 U/L</th>
<th>≥ 50 U/L</th>
<th>≥ 3 kJ/L</th>
<th>200 mg/dL (5.2 mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good's buffer</td>
<td>6.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Aminoantipyrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>(CHE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol oxidase</td>
<td>(CHO)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Peroxidase</td>
<td>(POD)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Standard</td>
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<td></td>
</tr>
</tbody>
</table>

Storage Instructions and Reagent Stability

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

**Note:** It has to be mentioned, that the measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.3 at 546 nm.

Warnings and Precautions

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


3. In very rare cases, samples of patients with gammapathy might give falsified results [8].

4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.

5. 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.

6. For professional use only!
Calculation
With standard or calibrator

Cholesterol [mg/dL] = \( \frac{A_{\text{Sample}}}{A_{\text{Std}}} \times \text{Conc. Std/Cal [mg/dL]} \)

Conversion factor
Cholesterol [mg/dL] x 0.02586 = Cholesterol [mmol/L]

Calibrators and Controls
For calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. The assigned values of the calibrator have been made traceable to the reference method gas chromatography-isotope dilution mass spectrometry (GC-IDMS).

Cat. No. | Kit size | \( x \) | \( x \) mL
---|---|---|---|
TruCal U | 5 9100 99 10 063 | 20 | 3 mL
| 5 9100 99 10 064 | 6 | 3 mL
TruLab N | 5 9000 99 10 062 | 20 | 5 mL
| 5 9000 99 10 061 | 6 | 5 mL
TruLab P | 5 9050 99 10 062 | 20 | 5 mL
| 5 9050 99 10 061 | 6 | 5 mL
TruLab L Level 1 | 5 9020 99 10 065 | 3 | 3 mL
TruLab L Level 2 | 5 9030 99 10 065 | 3 | 3 mL

Performance Characteristics
Measuring range
The test has been developed to determine cholesterol concentrations within a measuring range from 3 – 750 mg/dL (0.08 – 19.4 mmol/L). When values exceed this range samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

Specificity/Interferences
No interference was observed by ascorbic acid up to 5 mg/dL, bilirubin up to 20 mg/dL, hemoglobin up to 200 mg/dL and lipemia up to 2,000 mg/dL triglycerides.

For further information on interfering substances refer to Young DS [7].

Sensitivity/Limit of Detection
The lower limit of detection is 3 mg/dL (0.08 mmol/L).

Precision (at 37°C)

<table>
<thead>
<tr>
<th>Intra-assay precision</th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>108</td>
<td>1.76</td>
<td>1.62</td>
</tr>
<tr>
<td>Sample 2</td>
<td>236</td>
<td>1.45</td>
<td>0.61</td>
</tr>
<tr>
<td>Sample 3</td>
<td>254</td>
<td>1.57</td>
<td>0.62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-assay precision</th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>104</td>
<td>1.19</td>
<td>1.14</td>
</tr>
<tr>
<td>Sample 2</td>
<td>211</td>
<td>2.57</td>
<td>1.22</td>
</tr>
<tr>
<td>Sample 3</td>
<td>245</td>
<td>2.28</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Method Comparison
A comparison of DiaSys Cholesterol FS (y) with a commercially available test (x) using 78 samples gave following results:

\( y = 1.00 x – 2.50 \text{mg/dL}; r = 0.995 \)

Reference Range [5]
Desirable \( \leq 200 \text{mg/dL (5.2 mmol/L)} \)
Borderline high risk 200 – 240 mg/dL (5.2 – 6.2 mmol/L)
High risk \( > 240 \text{mg/dL (6.2 mmol/L)} \)

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

Clinical Interpretation
The European Task Force on Coronary Prevention recommends to lower TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L) [2].

Literature

Manufacturer
DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany

The European Task Force on Coronary Prevention recommends to lower TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L) [2].