Phospholipids FS

Diagnostic reagent for quantitative in-vitro determination of choline-containing phospholipids in serum and plasma on photometric systems

Order information
Cat. No. Kit size
1 5741 99 10 930 R1 4 x 20 mL + R2 2 x 10 mL
1 5740 99 10 041 3 x 1 mL Standard

Summary [2,3]
Phospholipids are a major component of all biological lipid bilayer membranes. Phosphatidyl choline (known as lecithin), lysophosphatidyl choline and sphingomyelin make up 95% of the phospholipids in human blood plasma. Certain plasma phospholipid values seem to be affected by pathological conditions like liver disease, coronary heart disease and diabetes. Furthermore, some diseases are characterized by altered phospholipid concentrations or compositions in blood plasma, like obstructive jaundice, Tangier disease, hypobeta-lipoproteinemia or LCAT deficiency.

Method
Enzymatic colorimetric test

Principle
Phosphatidyl choline + H₂O → Phospholipase D > Choline + Phosphatic acid
Choline + 2 O₂ + H₂O → Choline oxidase > Betaine + 2 H₂O₂
2 H₂O₂ + 4-Aminoantipyrine + TBHBA → Peroxidase > Chinone dye + 4 H₂O

Reagents
Components and Concentrations
R1: Tris buffer pH 8.0 75 mmol/L
CHBA 3 mmol/L
Choline oxidase ≥ 3 kU/L
R2: Tris buffer pH 8.0 75 mmol/L
4-Aminooantipyrine 6 mmol/L
Peroxidase ≥ 30 kU/L
Phospholipase D ≥ 3.0 kU/L
Standard: 4 mmol/L

Storage Instructions and Reagent Stability
The reagents 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.

Warnings and Precautions
1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes!
2. The reagents contain biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammapathy might give falsified results [6].
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!

Waste management
Please refer to local legal requirements.

Reagent Preparation
The reagents are ready to use. Avoid formation of foam. Do not shake the reagents.

Materials required but not provided
NaCl solution 9 g/L
General laboratory equipment

Specimen
Serum and plasma
Stability [4]
5 days at 20 – 25°C
1 month at 2 – 8°C
1 month at −20°C
Discard contaminated specimen! Only freeze once!

Assay Procedure
Application sheets for automated systems are available on request.
Wavelength 570 nm
Optical path 1 cm
Temperature 37°C
Measurement against reagent blank

<table>
<thead>
<tr>
<th>Sample/standard</th>
<th>Blank</th>
<th>Sample/standard</th>
<th>Sample/standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dist. water</td>
<td>10 µL</td>
<td>10 µL</td>
<td>10 µL</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>1200 µL</td>
<td>1200 µL</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>300 µL</td>
<td>300 µL</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes. Read absorbance A1, then add:

∆A = (A2 – A1) sample/standard

Calculation
With standard
Phospholipids [mg/dL] = \( \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std.}} \times \text{Conc. Std. [mg/dL]} \)

Conversion factor
Phospholipids [mg/dL] x 0.0129 = Phospholipids [mmol/L]

Calibrators and Controls
For calibration, DiaSys TruCal Lipid or DiaSys Phospholipids Standard FS is recommended. The assigned values of the calibrator or standard are traceable to a primary standard material. DiaSys TruLab L should be assayed 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>
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<tbody>
<tr>
<td>TruCal Lipid</td>
<td>1 3570 99 10 045 3 x 2 mL</td>
</tr>
<tr>
<td>TruLab L Level 1</td>
<td>5 9020 99 10 065 3 x 3 mL</td>
</tr>
<tr>
<td>TruLab L Level 2</td>
<td>5 9030 99 10 065 3 x 3 mL</td>
</tr>
</tbody>
</table>
Performance characteristics

Measuring Range
The test has been developed to determine phospholipid concentrations within a measuring range from 0.09 – 13.3 mmol/L (7 – 1030 mg/dL). When values exceed this range, samples should be diluted 1+1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences
No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 60 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 2000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection
The lower limit of detection is 0.09 mmol/L (7 mg/dL).

Precision

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>Mean [mmol/L]</th>
<th>SD [mmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.62</td>
<td>0.01</td>
<td>1.44</td>
</tr>
<tr>
<td>Sample 2</td>
<td>3.20</td>
<td>0.03</td>
<td>1.09</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.36</td>
<td>0.03</td>
<td>1.03</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
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<th>SD [mmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.99</td>
<td>0.04</td>
<td>3.83</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.59</td>
<td>0.04</td>
<td>2.75</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.80</td>
<td>0.08</td>
<td>2.82</td>
</tr>
</tbody>
</table>

Method Comparison
A comparison of DiaSys Phospholipids FS (y) with a commercially available test (x) using 110 serum and plasma samples gave following results:

\[ y = 0.830 x + 0.163 \text{ mmol/L; } r = 0.996 \]

Reference Range [1]

<table>
<thead>
<tr>
<th></th>
<th>mmol/L</th>
<th>mg/dL</th>
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</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>0.90 – 2.19</td>
<td>70 – 170</td>
</tr>
<tr>
<td>Infant</td>
<td>1.29 – 3.55</td>
<td>100 – 275</td>
</tr>
<tr>
<td>Child</td>
<td>2.32 – 3.81</td>
<td>180 – 295</td>
</tr>
<tr>
<td>Adult</td>
<td>1.61 – 3.55</td>
<td>125 – 275</td>
</tr>
</tbody>
</table>

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

Literature
4. Data on file at DiaSys Diagnostic Systems GmbH.

Manufacturer
DiaSys Diagnostic Systems GmbH
Alte Strasse 9   65558 Holzheim   Germany