Diagnostic reagent for quantitative in vitro determination of Lp-PLA₂ (Lipoprotein-associated phospholipase A₂) in serum and plasma on photometric systems

Order Information
Cat. No. Kit size
171819910936 R1 1 x 20 mL + R2 1 x 4.75 mL + R3 1 x 0.25 mL
171819910937 R1 1 x 10 mL + R2 1 x 3.8 mL + R3 1 x 0.2 mL

Summary [1-4]
Lipoprotein-associated phospholipase A₂ (Lp-PLA₂), also known as platelet-activating factor acetylhydrolase (PAF-AH), is a calcium-independent phospholipase released by inflammatory cells in atherosclerotic plaques. In circulation, Lp-PLA₂ is predominantly associated with LDL particles whereas only a small portion of enzyme is associated with HDL. Lp-PLA₂ hydrolyzes oxidized LDL to generate two pro-atherogenic and pro-inflammatory compounds: Lyso phosphatidylcholine (lyso-PC) and oxidized free fatty acids (oxFFA). Both substances play a major role in the development of vulnerable atherosclerotic plaques. Concentration of Lp-PLA₂ is independent of the presence of other cardiovascular risk factors, shows minimal biovariability and is not elevated in systemic inflammatory reactions. Lp-PLA₂ is a beneficial indicator for cardiovascular disease (CVD) risks, and may represent a potential therapeutic target for the reduction of such risks.

Method
UV test using 1-myristoyl-2-(4-nitrophenylsuccinyl)-sn-glycero-3-phosphocholine.

Principle
Lp-PLA₂ hydrolyzes the sn-position of the substrate 1-myristoyl-2-(4-nitrophenylsuccinyl)-sn-glycero-3-phosphocholine producing 4-nitrophenylsuccinate. After degradation in aqueous solution, 4-nitrophenol develops which can be detected photometrically; Lp-PLA₂ activity is determined by a change in absorbance at the defined wavelengths.

Reagents
Components and Concentrations
R1: Buffer pH 7.6 < 500 mmol/L EDTA < 50 mmol/L
R2: Buffer pH 2.7 < 200 mmol/L
R3: Alcohol 99% 1-myristoyl-2-(4-nitrophenylsuccinyl-)sn-glycero-3-phosphocholine < 200 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 and contamination is avoided. Do not freeze the reagents!

Warning and Precautions
2. In very rare cases, samples of patients with gammopathies might give falsified results [6].

3. 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.

4. For professional use only!

Waste Management
Please refer to local legal requirements.

Reagent Preparation
Reagent 2 and reagent 3 must be premixed before use. Due to hygroscopic components, reagent 3 shall be stored tightly closed, and should not stand open for longer than 5 min. Bring reagents to room temperature before mixing.

Mix very gently to avoid foaming. In case of precipitation, leave premixed reagent until it is completely homogenized.

Stability of premixed R2/R3: 8 weeks if stored at 2 – 8°C.

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

Specimen
Serum, heparin plasma or EDTA plasma

Stability [5]:
4 weeks at 2 – 8°C
2 days at 20 – 25°C
3 months at −20°C

Freeze only once! Discard contaminated specimens!

Assay Procedure
Application sheets for automated systems are available on request.

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Optical path</th>
<th>Temperature</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>405 nm/505 nm</td>
<td>1 cm</td>
<td>37°C</td>
<td>Against reagent blank</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cat. No</th>
<th>Reagent volume R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 7181 99 10 936</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>1 7181 99 10 937</td>
<td>0.20 mL</td>
</tr>
</tbody>
</table>

| Sample or calibrator | 10 µL |
| Dist. water | 10 µL |
| Reagent 1 | 1000 µL |
| Mix, incubate approx. 5 min, then add: |
| Reagent 2 | 250 µL |
| Mix, read absorbance after 2 min and start stop watch. Read absorbance again exactly after 1, 2 and 3 minutes. |

Calculation
With calibrator
\[
Lp - PLA₂ [U/L] = \frac{\Delta A/\text{min. Sample}}{\Delta A/\text{min. Cal.}} \times \text{Conc. Cal. [U/L]}
\]
Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal Lipid calibrator is recommended. The method is traceable to the molar extinction coefficient of 4-nitrophenol. For internal quality control, DiaSys TruLab L Level 1 and Level 2 should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

**Note:** For reconstitution of TruLab L Level 2 add exactly 1 mL of distilled water. Reconstitution of TruLab L Level 1 should be done according to the instruction supplied with the product. **Replacement labels are attached to the reagent kit to identify TruLab L Level 2 with reduced reconstitution volume.**

<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

**Measuring range**
The test has been developed to determine Lp-PLA₂ activities from 50 U/L up to 2000 U/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 50 mg/dL, bilirubin up to 50 mg/dL, hemoglobin up to 1000 mg/dL, and lipemia up to 2000 mg/dL triglycerides.

**Sensitivity/Limit of Detection**
The lower limit of detection is 10 U/L.

**Precision**

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>Mean [U/L]</th>
<th>SD [U/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>319</td>
<td>2.02</td>
<td>0.63</td>
</tr>
<tr>
<td>Sample 2</td>
<td>633</td>
<td>4.40</td>
<td>0.69</td>
</tr>
<tr>
<td>Sample 3</td>
<td>1113</td>
<td>7.98</td>
<td>0.72</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total precision</th>
<th>Mean [U/L]</th>
<th>SD [U/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI n = 80</td>
<td>Sample 1</td>
<td>314</td>
<td>4.80</td>
</tr>
<tr>
<td>Sample 2</td>
<td>625</td>
<td>10.0</td>
<td>1.61</td>
</tr>
<tr>
<td>Sample 3</td>
<td>1105</td>
<td>13.3</td>
<td>1.20</td>
</tr>
</tbody>
</table>

**Method Comparison**
A comparison of DiaSys Lp-PLA₂ FS (y) with an activity test (x) using 97 samples gave following results:
\[ y = 0.909 \times x - 4.28 \text{ U/L}; r = 0.999 \]

**Reference Range**

| Adults | Men | < 639 U/L |
|        | Women | < 507 U/L |

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

**Literature**

5. Personal communication from Prof. Dr. med. Karl Winkler, Universitätsklinikum Freiburg, Germany

**Manufacturer**

DiaSys Diagnostic Systems GmbH
Alte Strasse 9   65558 Holzheim   Germany