Product Name: Anti-Lysobisphosphatidic Acid (LBPA) Human IgG ELISA

Product Number: K-4900

Kit Components:

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-4901</td>
<td>Anti-LBPA Coated Plate</td>
<td>1 plate</td>
</tr>
<tr>
<td>K-4902</td>
<td>Anti-LBPA Standards (set of 5)</td>
<td>Set of 5, 0.5 mL each</td>
</tr>
<tr>
<td>K-4903</td>
<td>Sample Buffer</td>
<td>1 bottle (14 mL)</td>
</tr>
<tr>
<td>K-SEC5</td>
<td>Anti-Human IgG HRP</td>
<td>1 vial</td>
</tr>
<tr>
<td>K-PBSB</td>
<td>Diluent</td>
<td>1 bottle (14 mL)</td>
</tr>
<tr>
<td>K-PTAB</td>
<td>PBS Tablets</td>
<td>2 tablets</td>
</tr>
<tr>
<td>K-TMB1</td>
<td>TMB Solution</td>
<td>1 bottle (12 mL)</td>
</tr>
<tr>
<td>K-STOPT</td>
<td>1 N H₂SO₄ Stop Solution</td>
<td>1 bottle (10 mL)</td>
</tr>
<tr>
<td></td>
<td>Microtiter plate seal</td>
<td>2 seals</td>
</tr>
</tbody>
</table>

Each assay kit provides sufficient reagents for 96 assay points (including standards)

Researcher provides:
- Microtiter plate reader capable of reading absorbance at 450 nm.
- Pipettes (20 µL, 200 µL, and 1,000 µL)
- Reagent grade water
- Microcentrifuge tubes or similar for sample dilution

Storage:
UPON RECEIPT STORE KIT AT 4 °C. Under proper storage conditions, the kit components are stable for 6 months from date of receipt. Allow the reagents to warm to room temperature before opening vials.

Background:
Lysobisphosphatidic acids (LBPAs), also known as bis-(monoacylglycerol)phosphates (BMPs) are specialized lipid molecules reported to play a role in intracellular protein and lipid transport in healthy cells. Their accumulation was observed in pathologic liver tissue, and it is believed that LBPA also serve as antigens for auto-antibody generation in a human autoimmune condition termed antiphospholipid syndrome (APS). APS is a well-studied but incompletely understood disorder that is often associated with recurrent pregnancy loss and increased risk for pathologic embolism.

Echelon’s Anti-LBPA Human IgG ELISA measures the amount of anti-LBPA IgG antibody in serum. Anti-LBPA antibodies are determined using a standard curve generated from human plasma with known amounts of anti-LBPA. The design of the Echelon’s Anti-LBPA Human IgG ELISA allows researchers to study anti-LBPA binding with or without associated co-factor proteins. Echelon’s Anti-LBPA Human IgG ELISA is a robust and simple method to measure anti-LBPA in serum samples.

References:
**Suggested Protocol** (for duplicate points of standards and samples):

1. Bring assay kit to room temperature.

2. Prepare 1X Washing Buffer by dissolving each PBS tablet with 200 mL reagent grade water.

3. Dilute serum samples with Assay Buffer (K-4903) by mixing 297 µL Assay Buffer with 3 µL of serum samples (1:100 dilution). If desired, co-factor protein can be added at this step.

4. Remove Anti-LBPA Coated Plate (K-4901) from plastic bag. Add 100 µL/well of the Anti-LBPA Standards (K-4902, no dilution needed) or the diluted serum samples (step 3). Cover with plate seal and incubate at room temperature for 30 minutes. Suggested detection plate layout is shown below.

5. After incubation, discard the standards, control and samples. Wash plate 3 times with 200 µL/well of washing buffer (step 2). **Do not use automatic plate washer.**

6. Dilute 12 µL of the anti-human IgG HRP (K-SEC5) with 12 mL Diluent (K-PBSB). Mix well. Add 100 µL/well of the diluted anti-human IgG HRP solution. Cover with new plate seal and incubate at room temperature for 1 hour.

7. After incubation, discard the anti-human IgG HRP. Wash plate 3 times with 200 µL/well of washing buffer (step 2). **Do not use automatic plate washer.**

8. Add 100 µL/well of the TMB solution. Let blue color develop at room temperature for approximately 30 minutes.

9. Stop with 50 µL/well of the 1 N H₂SO₄ Stop Solution (K-STOPt). Read plate and record absorbance at 450 nm.

10. Analyze data using linear regression for the standard curve. The Anti-LBPA Standards’ GPL units are listed on vials.

**Assay Notes:**

1. Automatic plate washer maybe too aggressive.

2. Standards GPL unit was calibrated using Louisville APL Diagnostics In.’s reference LAPL-GM200G.

---

Echelon Biosciences products are sold for research and development purposes only and are not for diagnostic use or to be incorporated into products for resale without written permission from Echelon Biosciences. Materials in this publication, as well as applications and methods and use, may be covered by one or more U.S. or foreign patents or patents pending. We welcome inquiries about licensing the use of our trademarks and technologies at busdev@echelon-inc.com.

TDS K-4900 Rev: 1 (2/10/10)