Cardiolipin IgG
ELISA Kit

Catalog Number KA0941
96 assays
Version: 06

Intended for research use only
# Table of Contents

**Introduction** ............................................................................................................. 3

- Intended Use .............................................................................................................. 3
- Background ................................................................................................................ 3
- Principle of the Assay ............................................................................................... 3

**General Information** ............................................................................................... 4

- Materials Supplied .................................................................................................... 4
- Storage Instruction ..................................................................................................... 4
- Materials Required but Not Supplied ........................................................................ 4
- Precautions for Use .................................................................................................... 4

**Assay Protocol** .......................................................................................................... 6

- Reagent Preparation ................................................................................................... 6
- Sample Preparation .................................................................................................... 6
- Assay Procedure ......................................................................................................... 6

**Data Analysis** ........................................................................................................... 7

- Calculation of Results .............................................................................................. 7
- Performance Characteristics ...................................................................................... 8

**Resource** .................................................................................................................. 9

- Reference .................................................................................................................. 9
- Plate Layout ............................................................................................................... 10
Introduction

Intended Use

The Cardiolipin IgG ELISA Kit is intended for the detection of IgG antibody to Cardiolipin in human serum or plasma.

Background

Measurement of IgG, IgM and IgA cardiolipin autoantibodies (aCL) by EIA is the standard procedure for the detection of antiphospholipid antibodies (aPL) in persons with suspected antiphospholipid syndrome (APS). High aCL concentrations are associated with increased risk of venous and arterial thrombosis, recurrent pregnancy loss and thrombocytopenia. Persons with the anti-cardiolipin syndrome have one of the above features and have antibodies to cardiolipin and/or a positive lupus anticoagulant test. The antibodies present to cardiolipin may be of the IgG, IgA, IgM isotypes. Testing for the various antibody isotypes to cardiolipin aid in diagnosis of the anti-phospholipid syndrome in persons with SLE or lupus-like disorders. Binding of aCL to CL in persons with autoimmune diseases is dependent on the presence of the cofactor beta-2-glycoprotein I (beta2-GPI); this binding is independent of beta-2-GPI in persons with infectious diseases (e.g., syphilis, tuberculosis). Recognition of the role of beta-2- GPI in the binding of aCL led to development of assay for direct measurement of beta-2-GPI autoantibodies using beta-2-GPI as antigen, allowing a clear distinction between beta-2-GPI autoantibodies and those that bind to CL alone.

Principle of the Assay

Diluted serum is added to wells coated with purified aCL antigen. aCL specific IgG antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of specific antibody in the sample.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with Cardiolipin antigen</td>
<td>96 (12x8) wells</td>
</tr>
<tr>
<td>Sample Diluent (ready to use)</td>
<td>22 mL</td>
</tr>
<tr>
<td>Calibrator (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Positive Control (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Enzyme conjugate (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Wash concentrate 20X</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

✓ Store the kit at 2-8°C.
✓ Keep microwells sealed in a dry bag with desiccants.
✓ The reagents are stable until expiration of the kit.
✓ Do not expose test reagent to heat, sun or strong light.

Materials Required but Not Supplied

✓ Distilled or deionized water
✓ Precision pipettes
✓ Disposable pipette tips
✓ ELISA reader capable of reading absorbance at 450 nm
✓ Absorbance paper or paper towel
✓ Graph paper

Precautions for Use

✓ Warnings and Precautions

• Potential biohazardous materials:
The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious
agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.

- This kit is designed for research use only.
- Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

✓ Limitation of the test
- Lipemic or hemolyzed samples may cause erroneous results.
Assay Protocol

Reagent Preparation

Prepare 1X Wash buffer by adding the contents of the bottle (25 mL, 20X) to 475 mL of distilled or deionized water. Store at room temperature (20-25°C).

Sample Preparation

✓ Collect blood specimens and separate the serum.
✓ Specimens may be refrigerated at 2-8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Assay Procedure

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 μL of the sample to 200 μL of sample diluent. Mix well.
3. Dispense 100 μL of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 μL sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 μL of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 μL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 μL of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 μL of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 μL of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.
Data Analysis

Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

✓ Example of typical results:
   Calibrator mean O.D. = 0.8
   Calibrator Factor (CF) = 0.5
   Cut-off Value = 0.8 x 0.5 = 0.400
   Positive control O.D. = 1.2
   Ab Index = 1.2 / 0.4 = 3
   Sample O.D. = 1.6
   Ab Index = 1.6 / 0.4 = 4.0

✓ Quality Control

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be greater than 0.250
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should fall within the range specified on the COA/label.

✓ Interpretation

The following is intended as a guide to interpretation of aCL antibody test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

- Antibody Index Interpretation
  <0.9 No detectable aCL IgG antibody by ELISA.
  0.9-1.1 Borderline positive. Follow-up testing is recommended.
  >1.1 detectable aCL IgG antibody by ELISA.

- Converting of Ab Index to GPL

As an option, Ab index may be converted to GPL units by multiplying Ab index value by 11. GPL units may then be interpreted as follows:

  <10 GPL  Negative
  10-15 GPL  Borderline positive
  15-80 GPL  Low/Medium Positive
  >80 GPL  High Positive
Performance Characteristics

✓ Sensitivity and Specificity
291 sera sample were tested by ELISA and a reference ELISA methods. 118 sera were positive and 164 sera were negative by both methods. The agreement between the two methods was 96% (282/291). The results are summarized below:

<table>
<thead>
<tr>
<th></th>
<th>aCL Ab ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Reference ELISA Kit</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
</tr>
</tbody>
</table>

✓ Precision

- Intra-Assay Study

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1.22</td>
<td>0.09</td>
<td>6.55</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>0.78</td>
<td>0.05</td>
<td>6.41</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>0.22</td>
<td>0.02</td>
<td>9.09</td>
</tr>
</tbody>
</table>

- Inter-Assay Study

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1.17</td>
<td>0.1</td>
<td>8.54</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.84</td>
<td>0.09</td>
<td>10.8</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.27</td>
<td>0.04</td>
<td>14.8</td>
</tr>
</tbody>
</table>
Reference


## Plate Layout

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>