Cardiolipin IgG/IgM ELISA

For the qualitative and quantitative determination of IgG/IgM antibodies against cardiolipin in serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 35-CAGHU-E01
Size: 96 wells
1. Intended Use

**Cardiolipin-GM** is a solid phase enzyme immunoassay employing highly purified cardiolipin plus native human β2-glycoprotein I for the quantitative and qualitative detection of IgG and/or IgM antibodies against cardiolipin in human serum. Anti-cardiolipin antibodies mainly recognize specific epitopes on a complex composed of cardiolipin and β2-glycoprotein I which are only expressed when β2-glycoprotein I interacts with cardiolipin.

The assay is an aid in the diagnosis and risk estimation of thrombosis in patients with systemic lupus erythematosus (SLE).

2. Clinical Application and Principle of the Assay

Antibodies against cardiolipin belong to the group of anti-phospholipid antibodies specific for negatively charged phospholipids, components of biological membranes. Cardiolipin is an acidic phospholipid derived from glycerol and was named because of its isolation from bovine heart in 1941. Anti-phospholipid antibodies are frequently found in sera of patients with systemic lupus erythematosus (SLE) and related diseases. The prevalence of anti-cardiolipin antibodies in SLE is 24-50%.

The occurrence of anti-cardiolipin antibodies in patients with SLE and related diseases is typical for a secondary anti-phospholipid syndrome (APS). In contrast, anti-cardiolipin antibodies in patients with no other autoimmune diseases characterize the primary anti-phospholipid syndrome (APS). Many studies have shown a correlation between these autoantibodies and an enhanced incidence of thrombosis, thrombocytopenia and habitual abortions (as a consequence of placental infarct). The exact mechanism by which pathogenic anti-phospholipid antibodies induce thrombosis is not yet revealed fully.

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**Principle of the test**

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient’s antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.
3. Kit Contents

**To be reconstituted:**
- **5x Sample Buffer**: 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
  - Containing: Tris, NaCl, BSA, sodium azide < 0.1% (preservative)
- **50x Wash Buffer**: 1 vial, 20 ml - 50x concentrated (capped white: green solution)
  - Containing: Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)

**Ready to use:**
- **Negative Control**: 1 vial, 1.5 ml (capped green: colorless solution)
  - Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
- **Positive Control**: 1 vial, 1.5 ml (capped red: yellow solution)
  - Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
- **Cut-off Calibrator**: 1 vial, 1.5 ml (capped blue: yellow solution)
  - Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
- **Calibrators**: 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 GPL/ml or MPL/ml.
  - (color increasing with concentration: yellow solutions)
  - Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
- **Conjugates**: 1 vial, 15 ml IgG (capped blue: blue solution)
  - Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase
- **Conjugates**: 1 vial, 15 ml IgM (capped green: green solution)
- **TMB Substrate**: 1 vial, 15 ml (capped black)
  - Containing: Stabilized TMB/H2O2
- **Stop Solution**: 1 vial, 15 ml (capped white: colorless solution)
  - Containing: 1M Hydrochloric Acid

**Material required but not provided:**
- Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware(cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000ml). Microplate washing device (300 µl repeating or multi-channel pipette or automated system), adsorbent paper.

Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C, at least. **Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.**
5. Precautions of Use

5.1 Health hazard data

_This product is for in vitro diagnostic use only._ Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

**Recommendations and precautions**

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

**WARNING!** Calibrators, Controls and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or adsorbed by skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit.

Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation: We recommend test performance at 30°C/86°F for automated systems.**

Never expose components to higher temperature than 37°C/ 98.6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

_A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods._

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35-46°F up to three days, or frozen at -20°C/-4°F for longer periods.
7. Assay Procedure

### 7.2 Work flow

For pipetting scheme see Annex A, for the test procedure see Annex B. We recommend pipetting samples and calibrators in duplicate. Cut-off calibrator should be used for qualitative testing only.

**NOTE:** If IgG and IgM are determined in parallel, calibrators, controls and samples have to be done twice, for each subclass separately.

- Pipette 100 µl of each patient’s diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off calibrator and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.
8. Quantitative and Qualitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in **GPL/ml** or **MPL/ml (x-axis)**. For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in **GPL/ml** or **MPL/ml**.

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>Equivocal Range</th>
<th>Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 12 GPL/ml</td>
<td>12 - 18 GPL/ml</td>
<td>&gt;18 GPL/ml</td>
</tr>
<tr>
<td>&lt; 12 MPL/ml</td>
<td>12 - 18 MPL/ml</td>
<td>&gt;18 MPL/ml</td>
</tr>
</tbody>
</table>

**Example of a standard curve**

We recommend pipetting calibrators in parallel for each run.

<table>
<thead>
<tr>
<th>Calibrators IgG</th>
<th>OD 450/620 nm</th>
<th>CV % (Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 GPL/ml</td>
<td>0.066</td>
<td>3.2</td>
</tr>
<tr>
<td>3 GPL/ml</td>
<td>0.162</td>
<td>0.4</td>
</tr>
<tr>
<td>10 GPL/ml</td>
<td>0.291</td>
<td>1.7</td>
</tr>
<tr>
<td>30 GPL/ml</td>
<td>0.597</td>
<td>1.3</td>
</tr>
<tr>
<td>100 GPL/ml</td>
<td>1.101</td>
<td>2.9</td>
</tr>
<tr>
<td>300 GPL/ml</td>
<td>2.039</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Example of calculation**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Replicate (OD)</th>
<th>Mean (OD)</th>
<th>Result (GPL/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 01</td>
<td>0.772/0.752</td>
<td>0.762</td>
<td>48.8</td>
</tr>
<tr>
<td>P 02</td>
<td>1.058/1.038</td>
<td>1.048</td>
<td>82.9</td>
</tr>
</tbody>
</table>

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

**Do not use this example for interpreting patients results!**

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

For qualitative interpretation read the optical density of the cut-off calibrator and the patient samples. Compare patient’s OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

**Negative:** \[ \text{OD}_{\text{patient}} < 0.8 \times \text{OD}_{\text{cut-off}} \]

**Equivocal:** \[ 0.8 \times \text{OD}_{\text{cut-off}} \leq \text{OD}_{\text{patient}} \leq 1.2 \times \text{OD}_{\text{cut-off}} \]

**Positive:** \[ \text{OD}_{\text{patient}} > 1.2 \times \text{OD}_{\text{cut-off}} \]
9. Technical Data

Sample material: serum
Sample volume: 10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time: 90 minutes at 20-32°C/68-89.6°F
Calibration range: 0-300 GPL/ml or MPL/ml
Analytical sensitivity: 1.0 GPL/ml or MPL/ml
Storage: at 2-8°C/35-46°F, use original vials, only
Number of determinations: 96 tests

10. Performance Data

10.1 Analytical sensitivity
Testing sample buffer 30 times on Cardiolipin-GM gave an analytical sensitivity of 1.0 GPL/ml or MPL/ml.

10.2 Specificity and Sensitivity
The microplates are coated with highly purified cardiolipin and native human β2-glycoprotein I. No crossreactivities to other autoantigens have been found. Cardiolipin antibodies are detected in up to 70% of SLE patients (2). The data has been acquired with the Cardiolipin-GM.
10.3 Linearity
Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Dilution Factor</th>
<th>measured concentration (GPL/ml)</th>
<th>expected concentration (GPL/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/100</td>
<td>63.1</td>
<td>68.0</td>
<td>93.0</td>
</tr>
<tr>
<td>1</td>
<td>1/200</td>
<td>33.7</td>
<td>34.0</td>
<td>99.1</td>
</tr>
<tr>
<td>1</td>
<td>1/400</td>
<td>15.9</td>
<td>17.0</td>
<td>93.5</td>
</tr>
<tr>
<td>1</td>
<td>1/800</td>
<td>9.0</td>
<td>8.5</td>
<td>105.9</td>
</tr>
<tr>
<td>2</td>
<td>1/100</td>
<td>138.6</td>
<td>141.8</td>
<td>97.7</td>
</tr>
<tr>
<td>2</td>
<td>1/200</td>
<td>70.1</td>
<td>70.9</td>
<td>98.9</td>
</tr>
<tr>
<td>2</td>
<td>1/400</td>
<td>33.2</td>
<td>35.5</td>
<td>93.5</td>
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<tr>
<td>2</td>
<td>1/800</td>
<td>17.9</td>
<td>17.7</td>
<td>101.1</td>
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</tbody>
</table>

10.4 Precision
To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

<table>
<thead>
<tr>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Mean (GPL/ml)</td>
</tr>
<tr>
<td>1</td>
<td>586.2</td>
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<tr>
<td>2</td>
<td>67.4</td>
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<tr>
<td>3</td>
<td>34.5</td>
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</table>

10.5 Calibration
Because of the lack of WHO reference material, Cardiolipin-GM is calibrated against reference sera from N.E. Harris, Louisville. The results are expressed in GPL/ml for IgG and in MPL/ml for IgM. In addition, Cardiolipin-GM is standardized using the Sapporo-Standards HCAL for IgG and EY2C9 for IgM.

11. Literature

ANNEX A: Pipetting scheme

We suggest pipetting calibrators, controls and samples as follows:
For **quantitative interpretation** use calibrators to establish a standard curve.
For **qualitative interpretation** use cut-off calibrator.

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<td>CalA</td>
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<td>P1</td>
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<td>NC</td>
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<td>B</td>
<td>CalA</td>
<td>CalE</td>
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<td>NC</td>
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<td>C</td>
<td>CalB</td>
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<td>CalB</td>
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PC: positive control
NC: negative control
CC: Cut-off calibrator
P1: patient 1
P2: patient 2
P3: patient 3
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