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Product Information

Cardiolipin IgA ELISA kit

Catalog Number: EA100914
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The anti-Cardiolipin (aCL) IgA ELISA Kit is intended for the detection of IgA 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 patients with suspected antiphospholipid syndrome (APS). High aCL concentrations are associated with increased risk of venous and arterial thrombosis, recurrent pregnancy loss and thrombocytopenia. Patients with the anti-cardiolipin syndrome have one of the above clinical 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 aids in diagnosis of the anti-phospholipid syndrome in patients with SLE or lupus-like disorders. Binding of aCL to CL in patients 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 patients 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 Test

Diluted patient serum is added to wells coated with purified aCL antigen aCL specific IgA 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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Cardiolipin antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator 1 Vial (ready to use)	1.5 ml
4. Positive Control 1 vial (ready to use)	1.5 ml
5. Negative Control 1 vial (ready to use)	1.5 ml

6. Enzyme conjugate: 1 bottle (ready to use)	12 ml
7. TMB Substrate: 1 bottle (ready to use)	12 ml
8. Stop Solution: 1 bottle (ready to use)	12 ml
9. Wash concentrate 20X: 1 bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and Preparation

1. Collect blood specimens and separate the serum.
2. 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.

Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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.

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 a Standard Curve

Calibrator mean OD = 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
Patient 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. If 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 be greater than 1.2.

Interpretation

The following is intended as a guide to interpretation of aCL 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 IgA by ELISA.
  - 0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
  - >1.1 Detectable IgA IgG by ELISA.
- **Converting of Ab Index to APL**
  As an option, Ab index may be converted to APL units by multiplying Ab index value by 17. APL units may then be interpreted as follows:
  - <15 APL Negative
  - 15- 20 APL Borderline positive.
  - 21-80 APL Low/Medium Positive
  - > 80 APL High Positive

NOTE: Patient values above 80 APL should be reported as > 80 APL or retested after dilution. In case of dilution, final results must be multiplied by the dilution factor.

References

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