REFERENCES

1. Barka N, Reagan K, Agopian M, Peter JB. Frequency of anticardiolipin and antiphosphatidylserine antibodies in patients with suspected antiphospholipid syndrome. Clin Chem 1995;41:5.
2. Khamashta MA, Hughes GRV. Detection and importance of anticardiolipin antibodies. J Clin Pathol 1993;46:104-7.
3. Reyes H, Dearing L, Bick RL, Shoenfeld Y, Peter JB. Laboratory diagnosis of antiphospholipid syndromes. Lab Clin North Am 1995;15:85-108.
4. Harris EN, Pierangeli S. Anticardiolipin antibodies: specificity and function. Lupus 1994;3:217-22.
5. Barka N. Phospholipid autoantibodies - phosphatidylserine. In: Peter JB, Shoenfeld Y, editors. Autoantibodies. Amsterdam: Elsevier 1996:630-4.
6. Triplett DA. Assays for detection of antiphospholipid antibodies. Lupus 1994;3:281-7.
7. Khamashta MA, Cuadrado MJ, Mujic F, et al. The management of thrombosis in the antiphospholipid antibody syndrome. N Engl J Med 1995;332:993-7.
8. Olee T, Pierangeli S, Handley H, et al. A monoclonal IgG anticardiolipin antibody from a patient with the antiphospholipid syndrome is thrombogenic in mice. Proc Natl Acad Sci 1996;931:8606-11.
9. Hughes GRV. The antiphospholipid syndrome: ten years on. Lancet 1993;342:341-4.

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Calbiotech Inc.,

CALBIOTECH
A life science company

Cardiolipin IgA ELISA

Catalog No: CA035A (96 Tests)

INTENDED USE

The Calbiotech, Inc (CBI) anti-Cardiolipin (aCL) IgA ELISA Kit is intended for the detection of IgA antibody to Cardiolipin in human serum or plasma.

SUMMARY AND EXPLANATION OF THE TEST

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 aid 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 (*beta*$_2$-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.

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

MATERIALS 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
6. Graph paper

STORAGE AND STABILITY

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

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

SPECIMEN COLLECTION AND HANDLING

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

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).

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 100µl 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.

LIMITATIONS OF THE TEST

1. Lipemic or hemolyzed samples may cause erroneous results.