

CALDON BIOTECH INC. CARDIOLIPIN IgM ELISA

Catalog No. CA036M
(96 tests)

NAME AND INTENDED USE

The Caldon Biotech Inc. (CBI), anti-Cardiolipin (aCL) IgM ELISA is intended for use in evaluating patients with suspected autoimmune diseases.

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 (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified aCL antigen. aCL specific IgM 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 IgM antibody in the sample.

MATERIALS PROVIDED

- Microwell Strips: Cardiolipin Antigen coated wells (12 x 8 x 1 wells).
- Sample Diluent/Sorbent: 1 bottle (22 mL). Ready to use.
- Calibrator: Yellow Cap. (1.50 mL/vial). Ready to use.
- Positive Control: Red Cap. (1.50 mL/vial). Ready to use.
- Negative Control: Blue Cap. (1.50 mL/vial). Ready to use.
- Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
- TMB Substrate: 1 bottle (12 mL). Ready to use.
- Stop Solution: 1N H₂SO₄; 1 bottle (12 mL). Ready to use.
- Wash Concentrate: 1 bottle (50 mL), 20X concentrate.

STORAGE AND STABILITY

- 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 reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. 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, as 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
2. 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.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

5. 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.

SPECIMEN COLLECTION AND HANDLING

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 of serum sample.

PREPARATION FOR ASSAY

1. Bring all specimens and kit reagents to room temperature (20–25 °C) and gently mix.
2. Prepare washing buffer by adding the contents of the bottle (50 mL, 20X Wash concentrate) to 950 mL of distilled or deionized water in one-liter container. Store at room temperature.

ASSAY PROCEDURE

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 control 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. Repeat washing three times with washing buffer.
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. Repeat washing three times with washing buffer.
7. Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 µL of 1N H₂SO₄ to stop reaction.
9. Read O.D. on ELISA reader at 450 nm within 30 minutes after adding stop solution.

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 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:

- If the O.D. of the Calibrator should be greater than 0.250.
- The Ab index for Negative control should be less than 0.9.
- The Ab index for Positive control should be greater than 1.2.

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.

1. Antibody Index Interpretation

<0.9 No detectable IgM antibody.

0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.

>1.1 Indicative of autoimmune disorder.

2. Converting of Ab Index to MPL

As an option, Ab index may be converted to MPL units by multiplying Ab index value by 17. MPL units may then be interpreted as follows:

<15 MPL Negative

15- 20 MPL Borderline positive

21-80 MPL Low/Medium Positive

> 80 MPL High Positive

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

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTIC**1. Sensitivity and Specificity**

291 patient sera were tested by this 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:

	aCL Ab ELISA		
	+	-	Total
Reference ELISA	118	5	
+ Kit	123	4	164
-	168		
Total	122	169	291

**2. Precision
Intra-Assay Study**

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	16	1.22	0.09	6.55
2	16	0.78	0.05	6.41
3	16	0.22	0.02	9.09

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	10	1.17	0.1	8.54
2	10	0.84	0.09	10.7
3	10	0.27	0.04	14.8

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;93:8606-11.
9. Hughes GRV. The antiphospholipid syndrome: ten years on. *Lancet* 1993;342:341-4. Jan-03