

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 kit	+	118	5	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

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Warning

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BIOQUANT

CARDIOLIPIN IgA ELISA

Catalog No: BQ035A (96 tests)

INTENDED USE

The anti-Cardiolipin (aCL) IgA ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgA class antibodies 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. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450nm

- Absorbance paper or paper towel

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

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, 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
- 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.

SPECIMEN COLLECTION AND HANDLING

- 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.

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 (18-26 °C).

PREPARATION FOR ASSAY

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

ASSAY PROCEDURE

- Place the desired number of coated strips into the holder.
- 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.
- 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.
- Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
- Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
- Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel
- Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
- Add 100 µl of stop solution.
- 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

- 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.
- Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
- 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 IgA antibody.
0.9-1.1	Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1	Detectable IgA antibody.

2. 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.

LIMITATIONS OF THE TEST

- 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.
- Lipemic or hemolyzed samples may cause erroneous results.