Cardiolipin IgA ELISA Kit

10/18

(Catalog # E4659-100, 96 assays; Store at 2-8^{0}C)

I. Introduction:

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 (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.

II. Application:

Detection of IgA antibody to Cardiolipin

III. Sample Type:

Human serum or plasma

IV. Kit Contents:

Components	E4659-100	Part No.
Microplate	12 strips x 8 wells	E4659-100-1
Sample Diluent	22 ml	E4659-100-2
Calibrator	1 ml	E4659-100-3
Positive Control	1 ml	E4659-100-4
Negative Control	1 ml	E4659-100-5
Enzyme conjugate	12 ml	E4659-100-6
TMB Substrate	12 ml	E4659-100-7
Stop Solution	12 ml	E4659-100-8
Wash Buffer (20X)	25 ml	E4659-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VI. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{o}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Buffer:** 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).

VII. Warning & 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."
- 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.

VIII. Sample Preparation and Storage:

Collect blood specimens & separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 7 days. Store frozen at (-20°C) for up to six month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well.

IX. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
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.

X. Calculation

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:
1. 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 fall within the range specified on the COA/label.

INTERPRETATION
The following is intended as a guide to interpretation of IgA antibody 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 antibody to IgA antibody by ELISA.
0.9 - 1.1	Borderline positive. Follow-up testing is recommend if clinically indicated.
> 1.1	Detectable antibody to IgA antibody by ELISA.

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

XI. RELATED PRODUCTS:

- Cardiolipin IgA, IgG, IgM ELISA Kit (E4658)
- Cardiolipin IgG ELISA Kit (E4660)
- Cardiolipin IgM ELISA Kit (E4661)
- Cardiolipin Assay Kit (Fluorometric) (K944)
- Cardiolipin probe (B1183)
- (R)-(+)-Etomoxir (B1104)
- Mitochondria Isolation Kit for Tissue & Cultured Cells (K288)
- Mitochondria/Cytosol Fractionation Kit (K256)

FOR RESEARCH USE ONLY! Not to be used on humans.