AngioMax Anticardiolipin IgA ELISA kit

Catalog Number EA1003-1

Introduction

Antiphospholipid antibodies (APA) are a family of autoimmune and alloimmune immunoglobulins (IgA, IgM, IgA, or mixtures) that recognize protein phospholipid complexes in *in vitro* laboratory test systems. APA are found in autoimmune diseases such as systemic lupus erythematosus (SLE) and autoimmune haemolytic anaemia. The antiphospholipid syndrome has become recognized as a major cause worldwide of thrombosis, thrombocytopenia and recurrent abortion (1, 2, 3). The APA can be detected by an ELISA method using cardiolipin as antigen.

Principal of the Assay

The AngioMax Anticardiolipin IgA ELISA kit is developed for detection of anticardiolipin antibodies IgA in human serum and plasma. This assay employs a quantitative sandwich enzyme immunoassay technique that measures anticardiolipin IgA in less than 2.5 hours. Samples and standards are incubated in cardiolipin coated and stabilized microplate, allowing anticardiolipin antibodies in the samples to react with the immobilized antigen. After removal of unbound proteins by washing, peroxidase labeled antibodies specific for human IgA are added forming complexes with the cardiolipin bound antibodies. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of a peroxidase substrate. Upon stopping the reaction, result is obtained by reading the absorbance of each well using a microplate reader and the concentration of anticardiolipin antibodies is derived from the calibrator curve.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Cardiolipin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with cardiolipin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Anticardiolipin IgA Calibrator (2x):** Human antibodies to cardiolipin in a buffered protein base (lyophilized).

- **Anti-Human IgA Peroxidase Conjugate (rabbit, 600x)**: A 600-fold concentrate (40 µl).
- **Sample Diluent (2x):** A 2-fold buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2 - 8^0C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2 - 8^0C. Store anticardiolipin IgA calibrator and anti-human IgA peroxidase conjugate at -20^0C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator at -20^0C.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes. Remove serum and assay. Samples require 1:50 dilution into Sample Diluent. Store serum at $<-20^0$C for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2,000 x g for 10 minutes and assay. Samples require 1:50 dilution into Sample Diluent (e.g. Add 5 µl of sample to 245 µl of Sample Diluent). Dilute sample down to the sensitive range of the assay when samples exceed the detection levels. Store samples at $<-20^0$C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Standard Curve:** Reconstitute the Anticardiolipin IgA Calibrator with 1.2 ml of Sample Diluent to generate a 2x stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Calibrator stock solution twofold with equal volume of Sample Diluent to produce 150, 75, 37.5, 18.75, and 9.38 APL. Sample Diluent serves as the zero standard (0 APL). Run samples in triplicate.

Standard Point	Dilution	Phospholipid Units (APL)
P1	Anticardiolipin IgA Calibrator (2x) + 1 part Sample Diluent	150.00
P2	1 part P1 + 1 part Sample Diluent	75.00
P3	1 part P2 + 1 part Sample Diluent	37.50
P4	1 part P3 + 1 part Sample Diluent	18.75
P5	1 part P4 + 1 part Sample Diluent	9.38
P6	Sample Diluent	0.00

- **Sample Diluent (2x):** Dilute the Sample Diluent 1:2 with reagent grade water.
- **Wash Buffer (10x):** Dilute 10-fold Wash Buffer 1:10 with reagent grade water.
- **Anti-Human IgA Peroxidase Conjugate (600x):** Dilute the conjugate 1:600 with Sample Diluent.

Assay Procedure

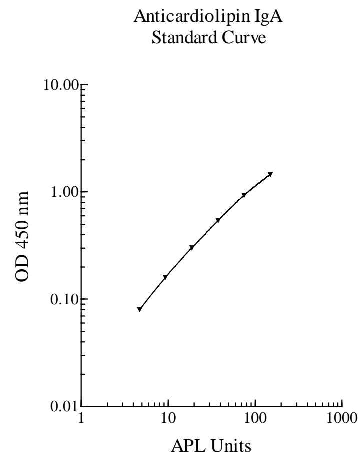
- Bring all reagents to room temperature (20 - 25^{0}C) before use. Prepare all reagents, working calibrators and samples as instructed.
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator at -20^{0}C.
- Add 50 µl of Sample Diluent each well and incubate for 30 minutes. Invert the plate and blot it on absorbent paper towel.
- Add 50 µl of the prepared calibrators and diluted samples to each well. Cover wells and incubate for 60 minutes at room temperature. Start the timer after the last sample addition.
- Wash four times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and blot it on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Anti-Human IgA Peroxidase Conjugate to each well and incubate for 30 minutes at room temperature. Turn on the microplate reader and set up the program in advance.
- Wash four times with 200 µl of Wash Buffer.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 to 20 minutes or till the optimal color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution per well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately.

Data Analysis

- Calculate the mean value of the triplicate readings for each calibrator and sample.
- To generate a standard curve, plot a log-log graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis of the log-log curve.
- Determine the unknown sample concentration from the x-axis. (Note: Do not multiply the sample value by 50, as both calibrator and sample are diluted 1:50. Only multiply the value by the extra dilution factor when samples exceed the detection levels.)

Standard Cur

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Interpretation of Results

High Positive: >80 APL
Medium Positive: 20 - 80 APL
Low Positive: 10 - 20 APL
Negative: <10 APL

References

1. Harris, E. N. (1990) *Br. J. Haematol.* 74:1
2. Harris, E. N. (1990) *Am. J. Clin. Pathol*. 94:476
3. Triplett, D.A. (1995) *Thrombosis Research* 78:1

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