Anti-Cardiolipin IgG Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIACGG1	Anti-Cardiolipin IgG Elisa	96 Tests

Intended Use:

The Cardiolipin IgG Elisa is intended for the detection and semi-quantitative determination of IgG antibodies to Cardiolipin in human sera or plasma. The assay is to be used to detect IgG antibodies in a single specimen. The results of the assay are to be used as an aid in the diagnosis of the anti-phospholipid syndrome in patients with autoimmune disease.

Summary and Principle:

Anti- Cardiolipin autoantibodies (ACA) are frequently found in patients with systemic lupus erythematosus (SLE). They are also found in patients with other autoimmune diseases, as well as in some individuals with no apparent previous underlying diseases. Elevated levels of ACA have been reported to be significantly associated with the presence of both venous and arterial thrombosis, thrombocytopenia, and recurrent fetal loss. Anti-phospholipid syndrome has been used to describe patients who present these clinical manifestations, inassociation with ACA or lupus anticoagulant. ACA are found in the immunoglobulin classes IgG, IgM and IgA. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune disease, whereas IgG antibodies will be found in progressive stages of manifested autoimmune disorders. ACA IgG shows a good correlation to the clinical status of the patient in thrombosis, thrombocytopenia, fetal loss, and some neurological disorders.
ACA IgA are often associated with IgG antibodies. ACA IgA seems to have a greater validity in thrombosis and fetal loss. Testing for ACA of various isotypes by ELISA aid in diagnosis of anti-phospholipid syndrome in patients with SLE and lupus-like disorders.
Purified Cardiolipin antigens are coated on the surface of microwells. Diluted patient serum or plasma, and calibrators, are added to the wells. The Anticardiolipin specific antibodies, if present, bind to the antigens. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibodies in the sample. The results are read by a microwell reader, and compared in a parallel manner with calibrators.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with Purified Cardiolipin Antigens. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 2 months at 2-8°C.
Calibrator set	6x1ml	6 vials containing Prediluted (1:101) Anti Cardiolipin antibodies with concentrations of 5, 10, 20, 40, 80, 160 GPL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable for 1 month at 2-8°C.
Control set	2x1ml	2 vials containing Negative and Positive controls (Prediluted). Values are indicated on the vial label. Once open stable for 1 month at 2-8°C. Ready to use.
Sample Diluent	50ml	1 vial containing 50ml of Sample Diluent made from Buffer. Store at 2-8°C. Once opened stable for 2 months at 2-8°C.
Wash Concentrate (20x)	50ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 950ml of distilled water before use. Once diluted it is stable at room temperature for two months.
TMB Substrate	12ml	*Amber bottle.* Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable for 2 months at 2-8°C.
Enzyme conjugate	12ml	*Red colour solution.* 1 vial containing 12ml of HRP labelled Anti Human IgG antibodies in Buffered saline. Once open, stable for 2 months at 2-8°C.
Stop Solution	12ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2-8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Specimen Collection:

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.

Storage and Stability:

1. Store the kit at 2 - 8°C.
2. After opening of pouch, the remaining coated wells must be carefully resealed inside the pouch with desiccants immediately. It is recommended to finish the whole coated wells within 30 days.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light during storage or usage.

Precautions and Safety:

1. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found nonreactive 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodiumazide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
5. To prevent injury and chemical burns, avoid contact with skin and eyes or inhalation and ingestion of the following reagents: Enzyme conjugate, TMB chromogenic substrate and Stop solution.

Procedure:

Reagent preparation:

Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 20x wash concentrate to make a final volume of 1 litre.
Bring all specimens and kit reagents to room temperature 20-25°C and gently mix.

STEP 1

Preparation: Place the desired number of coated strips into the holder. Pre-wash Coated wells – repeat washing three times with washing buffer.

STEP 2

Addition of the Diluent: Prepare 1:101 dilution of test samples by adding 5 ml of the sample to 500ml of sample diluent. Mix well.

STEP 3

Addition of the Sera, calibrators and controls: Dispense 100ml of diluted sera and prediluted calibrators and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well.

STEP 4

Incubation: Incubate for 30 minutes at room temperature.

STEP 5

Washing: Remove liquid from all wells. Repeat washing three times with washing buffer.

STEP 6

Addition of Enzyme Conjugate: Dispense 100ml of enzyme conjugate to each well.

STEP 7

Incubation: Incubate for 30 minutes at room temperature.

STEP 8

Washing: Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.

STEP 9

Addition of TMB Chromogenic Substrate: Dispense 100ml of TMB Substrate into each well and incubate for 15 minutes at room temperature.

STEP 10

Addition of Stop solution: Add 100ml of Stop Solution to stop reaction.
Make sure there are no air bubbles in each well before reading.

STEP 11

Measurement: Read O.D. at 450nm with a microwell reader.

Calculation of results:

1. Construct a standard curve by plotting O.D. 450nm on the y-axis against the concentration of calibrator GPL values on the x-axis on a log-log graph paper or log-in graph.
2. Using the O.D. value of each specimen, determine the concentration from the standard curve.
3. A typical example:

Calibrator Set	Cardiolipin IgG (GPL)	O.D. 450nm		O.D.450 nmMean	SD	CV %
Calibrator 1	5	0.267	0.240	0.254	0.019	7.531
Calibrator 2	10	0.443	0.456	0.450	0.009	2.045
Calibrator 3	20	0.826	0.835	0.831	0.006	0.766
Calibrator 4	40	1.220	1.159	1.190	0.043	3.626
Calibrator 5	80	1.612	1.650	1.631	0.027	1.647
Calibrator 6	160	2.021	2.035	2.028	0.010	0.488

Quality Control:

1. The negative control and positive control should be run with every batch of samples tested and the concentration must be within the range stated on its label.
2. The O.D. value of Sample Diluent (0 GPL) must be lower than 0.150 and the O.D. value of calibrator 160 GPL must be greater than 0.750.

Additional controls may be prepared from human serum specimens and kept below -20°C.

Interpretation of results:

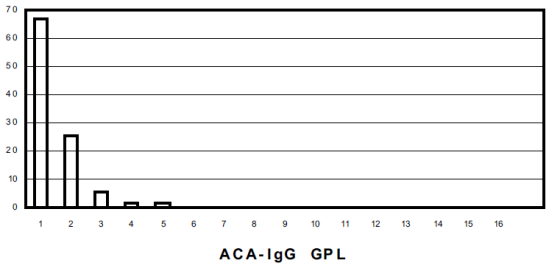
Negative:	< 10 GPL
Low positive:	10 – 19 GPL
Moderate positive:	20 – 79 GPL
High positive:	> 80 GPL

Expected Values:

Elevated levels of ACA are occasionally, though infrequently, observed in the normal population. However, several autoimmune and infectious diseases can result in transient or chronic increases in ACA.
Elevated ACA levels have been reported in SLA, rheumatoid arthritis, tuberculosis, Behcet's syndrome, and other illnesses 11,12,13,14.
The range of normal ACA values may vary from population to population.

Histogram

60 random normal samples are determined with Prestige Anti-Cardiolipin IgG Elisa.
The results of obtained mean values is 1.4 GPL. SD = 0.736.
Histogram of ACA IgG Total normal samples n=60



Limitation of the Test:

1. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
2. Although ACA has been associated with certain SLE subsets, the clinical significance of ACA in SLE and other diseases remains under investigation.

Performance Characteristics:

Sensitivity, specificity and accuracy:

A total of 72 samples from different sources were assayed with the Prestige Elisa ACA IgG test and with another commercially available Elisa test kit.

		Reference Elisa	
		N P	Total
Prestige ELISA	N P	27 (D) 18 (B) 0 (C) 27 (A)	45 27
Anti-Cardiolipin IgG	**TOTAL**	27	72
		45	

Relative sensitivity = A / (A+B) = 27 / (27 + 18) = 60 %
Relative specificity = D / (C+D) = 27 / (0 + 27) = 100%
Agreement = (A+D) / (A+B+C+D) = (27 + 27) / (27 + 18 +0 + 27) = 54 / 72 = 75%

Cross-reactivity:

A study was performed to determine the cross-reactivity of Prestige ACA IgG with other IgG antibodies. No cross-reactivity was found against the IgG positive samples of Rubella, CMV, HSV, EBV-VCA, Toxo, DS-DNA, Chlamydia trachomatis, ANA, Dengue and RF IgM.

Precision:

The mean, SD and % CV were calculated inter- and intra- assay:

Intra-assay	n	Mean MPL	SD	%CD
Serum 1	8	16.3	1.17	7.17
Serum 2	8	33.8	1.25	3.68
Serum 3	8	67.1	4.55	6.78
Inter-assay	**n**	**Mean MPL**	**SD**	**%CD**
Serum 1	8	16.5	1.39	7.94
Serum 2	8	35.9	2.17	6.04
Serum 3	8	69.4	2.83	4.07

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REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

CE

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