Anti Cardiolipin IgG/IgM GENLISA™ ELISA

REF : KBD301GM

Ver 4.0

IVD

Enzyme Immunoassay for Quantitative determination of Anti Cardiolipin IgG/IgM in serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

REF KBD301GM Σ 96 tests

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Introduction:

Cardiolipin is a negatively charged phospholipid which is typically located in the inner mitochondrial membrane[1]. Autoantibodies directed against cardiolipin are part of a group known as antiphospholipid antibodies which includes anti-β2 glycoprotein 1 autoantibodies. Measurement of anticardiolipin autoantibodies is considered to be one of the most important markers to support diagnosis of antiphospholipid syndrome (APS)[2,3].

APS is a systemic autoimmune disorder characterised by a combination of arterial and/or venous thromboses, pregnancy complications, such as recurrent foetal loss, together with elevated levels of antiphospholipid antibodies[4]. APS was first described in patients with systemic lupus erythematosus (SLE), though it has subsequently been established that SLE may be independent of an underlying disease[5].

APS can occur alone – Primary APS, or in association with other conditions, such as SLE – Secondary APS[6] (6). However, it has been demonstrated that anti-cardiolipin antibodies (aCLs) can be detected in patients with SLE that do not develop Secondary APS[7-9]. However, thromboembolic events are the most common clinical manifestation of APS

Anti-cardiolipin antibodies can recognise both cardiolipin and portions of the phospholipid-protein complex β2 glycoprotein 1-cardiolipin[10,11].

Studies have indicated an association of anti-cardiolipin IgG and IgM antibodies[2,7,11-16], with thrombotic events whereas others suggest these to be linked with IgG isotype, but not IgM[6,10,17].

aCL IgM antibodies have been shown to occur in infections such as chronic hepatitis C, leprosy, syphilis, but they are not directly involved in thrombotic events[17].

It is likely that the presence of antiphospholipid antibodies, including aCL IgG and IgM constitutes the single most recognisable risk factor in cases of recurrent pregnancy loss and late placenta-mediated obstetric complications[4-6,11,14-15,18,19]. Where patients can present with only adverse pregnancy outcomes with isolated vascular events or with both obstetric and thrombotic manifestations[6]. It has been suggested that anti-β2-glycoprotein I – cardiolipin antibodies are able to recognise the antigen on placental tissues, inhibiting the growth and differentiation of trophoblasts which may eventually cause defective placentation[20].

Intended Use:

The Anti-Cardiolipin IgG/IgM GENLISA™ ELISA is intended for the quantitative determination of Anti-Cardiolipin IgG/IgM in serum and plasma.

Principle:

Anti-Cardiolipin IgG/IgM GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay which is designed to quantitatively detect Anti-Cardiolipin IgG/IgM present in the human serum and plasma. Highly purified Cardiolipin Beta 2 Glycoprotein is pre-coated onto microwells. Samples and Controls are pipetted into microwells Anti-Cardiolipin IgG/IgM present in test sample binds to the antigen coated on the wells. And then enzyme labelled antibody conjugate is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of Anti-Cardiolipin IgG/IgM present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Coated Microtiter Plate (96 wells) – 1 no
2. Anti Cardiolipin IgG Standards (1.2 ml/vial) – 0, 5, 10, 20, 80 AU/ml
3. Anti Cardiolipin IgM Standards (1.2 ml/vial) – 0, 5, 10, 20, 80 AU/ml
4. IgG Positive Control – 1.2 ml
5. IgG Negative Control – 1.2 ml
6. IgM Positive Control – 1.2 ml
7. IgM Negative Control – 1.2 ml
8. Sample Diluent – 100 ml

9. Anti-human-IgG:HRP Conjugate - 15 ml
10. Anti-human-IgM: HRP Conjugate – 15 ml
11. (10X) Wash Buffer – 50 ml
12. TMB Substrate – 15 ml
13. Stop Solution –15 ml
14. Instruction Manual – 1 no

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
   Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Preparation of Samples:
All serum or plasma samples must be prediluted 1:100 with sample diluent; for example, 10 µL of sample should be diluted with 990 µL of sample diluent.

Reagent Preparation:

1. Allow all components to reach 2-8°C prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 50 ml of (10X) Wash Buffer to 450 ml of DI water. This is the working solution.

Test Procedure:

1. Add **100 ul Diluted Sample, Standards (IgG or IgM) , Positive Controls (IgG or IgM) and Negative Controls (IgG or IgM)** into appropriate wells.
2. Incubate at Room Temperature for **60 minutes**.
3. Aspirate and wash plate 3 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
4. Add **100 ul Anti-human-IgG:HRP Conjugate** or **Anti-human-IgM:HRP Conjugate** into each well. Mix well.
5. Incubate at Room Temperature for **60 minutes**.
6. Aspirate and wash plate 3 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7. Add **100 ul** of **TMB Substrate** to all wells.
8. Incubate at Room Temperature for 15 minutes.
9. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 5 minutes of stopping the reaction.

Calculation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Anti Cardiolipin IgG/IgM concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Anti Cardiolipin IgG/IgM Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a 4-PL (2^{nd} order), or cubic spline is best recommended for automated results.

Validity of the Test:

The test is valid is the controls fall in below given range:

Negative Control: < 16 AU/ml
Positive Control: >20 AU/ml

Measuring Range:
For assessment of IgG class antibodies
The assay measuring range (AMR) is 2 – 80 AU/mL.
Any value that reads below 2 AU/mL should be reported as "< 2 AU/mL". Any value that reads above 80 AU/mL should be reported as "> 80 AU/mL".

For assessment of IgM class antibodies
The assay measuring range (AMR) is 2.08 – 80 AU/mL.
Any value that reads below 2.09 AU/mL should be reported as "< 2.08 AU/mL". Any value that reads above 80 AU/mL should be reported as "> 80 AU/mL".

Metrology And Traceability:
For assessment of IgG class antibodies
The calibrators of this kit are traceable to the Centres for Disease Control (CDC) Human IgG Anti-Cardiolipin

Monoclonal Antibody IS2717.

For assessment of IgM class antibodies
The calibrators of this kit are traceable to the Centres for Disease Control (CDC) Human IgM Anti-Cardiolipin Monoclonal Antibody IS2718.

Interpretation of Results:

Concentration	Interpretation
< 8 AU/mL	The sample should be considered negative
8 – 10 AU/mL	The sample should be graded equivocal and repeat testing / sampling should be performed according to internal practices
>10 AU/mL	The sample should be considered Positive

Determination of a range of expected values for a “normal” population of a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore, each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population.

Positive results should be verified concerning the entire clinical status of the patient, with the decision for therapy being taken on an individual basis. It is recommended that each laboratory establishes its own normal and pathological ranges of Anti-Cardiolipin antibody values.

Performance Characteristics:
Representative performance data are shown. Results obtained at individual laboratories may vary.

Detection Capability:
For assessment of IgG class antibodies
The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation” using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	0.59 AU/mL
Limit of Detection (LoD)	1.25 AU/mL
Limit of Quantitation (LoQ)	2.00 AU/mL

For assessment of IgM class antibodies
The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation” using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	0.76 AU/mL
Limit of Detection (LoD)	1.45 AU/mL
Limit of Quantitation (LoQ)	2.08 AU/mL

Trueness:
For assessment of IgG class antibodies
Trueness of the Anti Cardiolipin Screen for assessment of IgG class antibodies has been demonstrated through performance of a recovery test using the CDC Human IgG Anti-Cardiolipin Monoclonal Antibody IS2717.

For assessment of IgM class antibodies
Trueness of the Anti Cardiolipin Screen for assessment of IgM class antibodies demonstrated through performance of a recovery test using the CDC Human IgM Anti-Cardiolipin Monoclonal Antibody IS2718.

Diagnostic Sensitivity and Specificity
For assessment of IgG class antibodies
The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 50 negative and 51 positive samples run on two reagent lots.

		Anti Cardiolipin IgG		Total
		Positive	Negative	
True State	Positive	47	4	51
	Negative	0	57	57
Total		47	61	108

Diagnostic sensitivity: 92%

Diagnostic specificity: 100%

For assessment of IgM class antibodies
The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 73 negative and 62 positive samples run on two reagent lots.

		Anti Cardiolipin IgG		Total
		Positive	Negative	
True State	Positive	50	12	62
	Negative	0	73	73
Total		50	85	135

Diagnostic sensitivity: 80%

Diagnostic specificity: 100%

Precision:
For assessment of IgG class antibodies
Precision of the Anti Cardiolipin Screen for determination of IgG class antibodies was determined by performing a complex precision study.

For assessment of IgM class antibodies
Precision of the Anti Cardiolipin Screen for determination of IgM class antibodies determined by performing a complex precision study.

Repeatability:
For assessment of IgG class antibodies
A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Data from one representative lot is shown below:

			Within run (Repeatability)	
Sample	n	Mean Conc. (AU/mL)	SD	CV%
1	75	6.95	0.38	5.5%
2	75	11.03	0.51	4.6%
3	75	20.12	0.94	4.7%
4	75	30.26	1.86	6.1%
5	75	50.11	2.58	5.1%
6	75	71.71	2.53	3.5%

For assessment of IgM class antibodies

A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Data from one representative lot is shown below:

			Within run (Repeatability)	
Sample	**n**	**Mean Conc. (AU/mL)**	**SD**	**CV%**
1	75	7.74	0.40	5.2%
2	75	12.46	0.51	4.1%
3	75	21.06	0.99	4.7%
4	75	32.07	1.46	4.6%
5	75	55.15	1.19	2.2%
6	75	75.45	2.33	3.1%

Reproducibility:
For assessment of IgG class antibodies
A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Results for the combined data from two lots is shown below:

			Within Laboratory (Reproducibility)	
Sample	**n**	**Mean Conc. (AU/mL)**	**SD**	**CV%**
1	150	6.98	0.49	7.0%
2	150	11.17	0.84	7.5%
3	150	20.16	1.87	9.3%
4	150	30.38	3.06	10.1%
5	150	50.76	5.02	9.9%
6	150	72.30	3.93	5.4%

For assessment of IgM class antibodies
A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators.
Results for the combined data from two lots is shown below:

			Within Laboratory (Reproducibility)	
Sample	**n**	**Mean Conc. (AU/mL)**	**SD**	**CV%**
1	150	7.65	0.46	6.0%
2	150	12.33	0.74	6.0%
3	150	21.03	1.60	7.6%
4	150	31.89	1.98	6.2%
5	150	54.28	2.70	5.0%
6	150	75.46	2.63	3.5%

Linearity:
For assessment of IgG class antibodies
Linearity was evaluated based on CLSI EP-06, “Evaluation of the Linearity of Quantitative Measurement Procedures”. For anti-cardiolipin IgG concentration by Anti Cardiolipin Screen, the measurement procedure shows linearity for the interval from 0.84 to 83.68 ng/mL within the allowable deviation of linearity (ADL) of ±15 %.

For assessment of IgM class antibodies
Linearity was evaluated based on CLSI EP-06, “Evaluation of the Linearity of Quantitative Measurement Procedures”. For anti-cardiolipin IgM concentration by Anti Cardiolipin Screen, the measurement procedure shows linearity for the interval from 0.82 to 86.88 AU/mL within the allowable deviation of linearity (ADL) of ±15 %.

Analytical Specificity:
For assessment of IgG class antibodies
The following substances do not interfere with a bias of >±15% in the Anti Cardiolipin Screen assay when assessing IgG class antibodies when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Billirubin Conjugated	15 mg/dL
Bilirubin Unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglycerides	500 mg/dL

For assessment of IgM class antibodies
The following substances do not interfere with a bias of >±15% in the Anti Cardiolipin Screen assay when assessing IgM class antibodies when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Billirubin Conjugated	15 mg/dL
Bilirubin Unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglycerides	500 mg/dL

Serum-plasma study:
For assessment of IgG class antibodies
The Anti Cardiolipin Screen matrix comparison study for assessment of IgG class antibodies was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 22 samples (18 native, 4 spiked) to cover the range were evaluated. Linear regression analysis was performed on the comparative data:

Sample Type	Slope (95% CI)	Intercept (ng/mL) (95% CI)	Correlation Coefficient (r)
SST	0.96 (0.92 to 0.98)	0.64 (-0.38 to 1.66)	1.00
Lithium Heparin	0.92 (0.88 to 0.96)	0.87 (-0.24 to 1.99)	1.00
Sodium Heparin	0.94 (0.89 to 0.98)	0.66 (-0.75 to 2.06)	0.99
EDTA	0.95 (0.92 to 0.99)	0.54 (-0.69 to 1.77)	1.00

For assessment of IgM class antibodies
The Anti Cardiolipin Screen matrix comparison study for assessment of IgM class antibodies was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 20 samples (16 native, 4 spiked) to cover the assay range were evaluated. Linear regression analysis was performed on the comparative data:

Sample Type	Slope (95% CI)	Intercept (ng/mL) (95% CI)	Correlation Coefficient (r)
SST	1.02 (0.94 to 1.09)	0.19 (-1.34 to 1.72)	0.99
Lithium Heparin	0.93 (0.82 to 1.04)	0.73 (-1.49 to 2.95)	0.97
Sodium Heparin	0.93 (0.84 to 1.01)	0.64 (-1.00 to 2.28)	0.98
EDTA	0.96 (0.86 to 1.05)	0.65 (-1.19 to 2.49)	0.98

LIMITATIONS OF USE

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays22. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
- The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	Add **100 ul Diluted Sample, Standards, Positive Controls and Negative Controls** into appropriate wells.
2	Incubate at Room Temperature for 60 minutes.
3	Aspirate and wash plate 3 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
4	Add **100 ul Anti-human-IgG:HRP Conjugate or Anti-human-IgM:HRP Conjugate** into each Mix well.
5	Incubate at Room Temperature for 60 minutes.
6	Aspirate and wash plate 3 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7	Add **100 ul** of **TMB Substrate** to all wells.
8	Incubate on shaker at Room Temperature for **15 minutes.**
9	Add **100 ul** of **Stop Solution**. **Read result with an ELISA reader at 450 nm** within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	Microtiter Plate (96 wells)
STD	Standards
POS CONT	Positive Control
NEG CONT	Negative Control
ENZY CONJ	Anti-human-IgG:HRP Conjugate
ENZY CONJ	Anti-human-IgM:HRP Conjugate
SAMP DIL	Sample Diluent
10X WASH BUF	(10X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature