Anti Cardiolipin IgA GENLISA® ELISA

REF : KBD301A

Ver 1.3

IVD

Enzyme Immunoassay for the Qualitative Determination of Anti Cardiolipin IgA antibody in human 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**

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REF KBD301A Σ 96 tests

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Introduction:

Anti-phospholipid syndrome (APS) is a systemic autoimmune disorder characterized by recurrent arterial or venous thrombosis and pregnancy morbidity. The condition is associated with the production of autoantibodies against phospholipid-binding proteins, particularly cardiolipin and β2-glycoprotein I. APS may occur as a primary condition or secondary to underlying autoimmune diseases such as systemic lupus erythematosus (SLE). Anti-cardiolipin antibodies of IgG and IgM classes form the laboratory criteria for APS; however, Anti Cardiolipin IgA has clinical value as an additional marker, particularly in patients with clinical features of APS who are negative for IgG and IgM antibodies (seronegative APS) and in patients with SLE. These antibodies contribute to a prothrombotic state by disrupting the balance between procoagulant and anticoagulant mechanisms, leading to manifestations such as deep vein thrombosis, stroke, myocardial infarction, recurrent miscarriage, and other vascular complications. Antiphospholipid antibodies are detected in a significant proportion of patients with SLE and may also be present at low frequency in the general population, with higher prevalence in young to middle-aged adults.

Intended Use:

The Anti Cardiolipin IgA GENLISA® ELISA is intended for the qualitative determination of Anti Cardiolipin IgA antibody in human serum and plasma.

Principle:

Anti Cardiolipin IgA GENLISA® ELISA is an indirect enzyme linked immnunosorbent assay which is designed to qualitatively detect Anti Cardiolipin IgA antibody present in the human serum and plasma. Purified ACA antigen is pre-coated onto microwells. Samples and Controls are pipetted into microwells and Anti Cardiolipin IgA antibody present in test sample binds to the antigen coated on the wells. And then enzyme labeled 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 (A and B) is added to microwells and color develops proportionally to the amount of Anti Cardiolipin IgA antibody present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. Negative Control - 1 ml
3. Positive Control - 1 ml
4. Enzyme Conjugate - 6.5 ml
5. (20X) Wash Buffer - 25 ml
6. Sample Diluent - 11 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

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.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

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.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 25 ml of Wash Buffer (20X) to 475 ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Sample Diluent** to the sample wells.
3. Add **5 ul Sample** to the respective sample wells except blank well. Mix gently.
4. Dispense **50 ul Positive Control** and **50 ul Negative Control** to the negative and positive wells respectively.
5. Shake gently for 30 seconds to mix well. Incubate at 37ºC for 20 minutes.
6. Aspirate and wash plate 5 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 **50 ul** of **Enzyme Conjugate** to each well except the blank well.
8. Incubate at 37ºC for 20 minutes.
9. Repeat the Aspiration / Wash Step (Step-6).
10. Add **100 ul** of **TMB Substrate** into each well except blank well.
11. Incubate at 37ºC for 10 minutes.
12. Add **100 ul** of **Stop Solution** into each well except blank well. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Cut-Off value (CO) = OD_{mean} of Negative Control x 3

Note: incase the OD_{mean} of Negative Control is < 0.100 then assume the same as 0.100

Positive Results: **OD value ≥ CO**
Specimens giving an absorbance equal to or greater than the CO are considered initially reactive, which indicates that Anti Cardiolipin IgA has probably been detected using Anti Cardiolipin IgA ELISA. All initially reactive specimens should be retested in duplicates using Anti Cardiolipin IgA ELISA before the final assay results interpretation. Repeatedly reactive specimens may be considered positive for Anti Cardiolipin IgA with Anti Cardiolipin IgA ELISA.

Negative Results: **OD value < CO**
Specimens giving absorbance less than the CO are negative for the assay, which indicates that no Anti Cardiolipin IgA has been detected with the Anti Cardiolipin IgA ELISA.

Positive	CO > 1.1
Equivocal	0.9 - 1.1
Negative	CO < 0.9

Criteria of Validation:

Anti Cardiolipin IgA results are considered to be valid, if
OD of Positive Control > Cut-Off Value

OD = Optical Density / Absorbance at 450nm

Reference Values:
It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method:
Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics: Sensitivity:
89.5%

Specificity:
The recombinant antigens used in the kit are specific for Anti-Cardiolipin Antibodies.

Precision:
Intra-Assay: CV% ≤15%. Inter-Assay: CV% ≤20%

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.

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **100 ul Sample Diluent 100 ul** to the **sample wells**.
3	Add **5 ul Sample** to the **respective sample wells except blank well**. Mix gently.
4	Dispense **50 ul Negative Control** and **50 ul Positive Control** to the negative and positive wells respectively.
5	Shake gently for 30 seconds to mix well. **Incubate** at **37ºC** for **20 minutes**.
6	Aspirate and **wash plate 5 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 **50 ul** of **Enzyme Conjugate** to each well except the blank well.
8	Seal the plate and **incubate** at **37ºC** for **20 minutes**.
9	Repeat the Aspiration / Wash Step.
10	Add **100 ul** of **TMB Substrate** into each well except blank well.
11	**Incubate** at **37ºC** for **10 minutes**.
12	Add **100 ul** of **Stop Solution into each well except blank well**. **Read result with an ELISA reader at 450 nm** within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	Microtiter Plate (8x12 wells)
POS CNTRL	Positive Control
NEG CNTRL	Negative Control
ENZY CONJ	Enzyme Conjugate
SAMP DIL	Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature