Anti Cardiolipin IgA GENLISA™ ELISA

REF : KBD301A

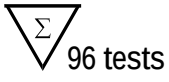
Ver 1.0

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**

For In-vitro Diagnostic Use only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN Pudgala LLP is strictly prohibited.

REF KBD301A 

Krishgen Pudgala LLP Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018. India.
Tel: +91-22-49198700 | email: sales@krishgenpudgala.com

Introduction:

The systemic autoimmune disorder which causes recurrent vascular thrombosis and pregnancy losses is Anti-phospholipid syndrome (APS). The pathogenesis of APS is production of auto antibodies to phospholipid protein. Anti-phospholipid syndrome is detected either by a positive Anti-Cardiolipin antibody (aCL) or lupus anticoagulant test. APS may be either primary or secondary; when APS is present in patients without any underlying clinical illness it is primary. Secondary APS occurs in patients with systemic lupus erythematosus (SLE) or any other underlying autoimmune disease.

The symptoms are observed by disturbing balance between procoagulant and anticoagulant factors and disruption of the clotting mechanism by the antiphospholipid antibodies (APLA) leading to leg ulcers, toe gangrene, myocardial infarction, purpura, stroke, recurrent miscarriage or preterm births. The autoantibodies are present in 50% of patients with SLE and 1-5% of the general population. The antiphospholipid antibodies are found in serum in 1% of healthy persons and 3% of older age group. Though APS can involve in any age group, the target group is 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. (40X) Wash Buffer - 20 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 2.5 ml of Wash Buffer (40X) to 97.5 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. 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.
10. Add **100 ul** of **TMB Substrate** to all wells.
11. Incubate at 37ºC for 10 minutes.
12. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance 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 ACA IgA has probably been detected using ACA IgA ELISA. All initially reactive specimens should be retested in duplicates using ACA IgA ELISA before the final assay results interpretation. Repeatedly reactive specimens may be considered positive for ACA IgA with ACA IgA ELISA.

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

Cut Off Value	OD_{mean} of Negative Control x 3
Positive	>= CO
Negative	< CO

Criteria of Validation:

ACA 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:
Limit of Detection: When detecting ACA IgA limits, laboratory quality control positive samples diluted till 1:8 with the ACA IgA ELISA kit should be in the positive.

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.

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.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Pudgala LLP shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Pudgala LLP shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Pudgala LLP be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Pudgala LLP, 2021.

THANK YOU FOR USING KRISHGEN PRODUCT!

Krishgen Pudgala LLP
Unit No.1/2, Om Sainath Commercial Complex,
Off Mankoli-Anjur Phata Road. Village Dapode, Bhiwandi 421302.

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**. 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** to all wells.
11	**Incubate** at **37ºC** for **10 minutes**.
12	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 (8x12 wells)
CTRL	Controls
CONJ	Enzyme Conjugate
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature