Phospholipid Screen IgG/IgM GENLISA™ ELISA

REF : KBD282

Ver 2.0

IVD

Enzyme Immunoassay for the Quantitative Determination of Phospholipid Screen IgG/IgM 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**

REF KBD282 **Σ 96 tests**

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Introduction:

The acronym "aPL" (anti-phospholipid antibodies) indicates improperly antibodies directed against phospholipids negatively charged like Cardiolipin (CL), Phosphatidyl serine (PS) Phosphatidyl inositol (PI) and phosphatidic acid (PA); more correctly the term anti-phospholipid antibodies indicate those antibodies directed against the complex between β2GPI and anionic phospholipids that can bind to the fifth domain of β2GPI. Among these, the Cardiolipin is the most commonly used phospholipid as an antigen for determining the aPL with ELISA method. The antibodies directed against the complex between β2GPI and negatively charged phospholipids, as Phosphatidyl serine (PS) Phosphatidyl inositol (PI) and phosphatidic acid (PA). Some researchers suggest the use of PS instead of Cardiolipin in ELISA assays, for a more precise diagnosis. However, these antibodies against phospholipids are less commonly used, even if their use may increase the clinical sensitivity of patients samples with suspected Anti-phospholipid Syndrome (APS), but it can' t replace the determination of autoantibodies anti-Cardiolipin.

Intended Use:

The Phospholipid Screen IgG/IgM GENLISA™ ELISA is intended for the quantitative determination of Phospholipid Screen IgG/IgM in human serum and plasma.

Principle:

Phospholipid Screen IgG/IgM GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay which is designed to quantitatively detect Phospholipid Screen IgG/IgM present in the human serum and plasma. Anti Phospholipid Screen test is based on the binding of antibodies in human serum directed against the antigenic complex between anionic phospholipids Beta 2 Glycoprotein these complex is pre-coated onto microwells. Standards, Samples and Controls are pipetted into microwells, Phospholipid Screen IgG/IgM present in test sample binds to the antigen coated on the wells. And then Anti-human-IgG:HRP conjugate or Anti-human-IgM:HRP 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 Phospholipid Screen 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. Standards Phospholipid Screen IgG/IgM (1.2 ml/vial) - 0, 5, 10, 20, 80 AU/ml
3. Negative Control – 1.2 ml
4. Positive Control – 1.2 ml
5. Anti-human-IgG:HRP Conjugate - 15 ml
6. Anti-human-IgM-:HRP Conjugate – 15 ml
7. (20X) Wash Buffer – 2 x 25 ml
8. Sample Diluent – 100 ml
9. TMB Substrate - 12 ml
10. Stop Solution - 12 ml
11. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Precision micropipettes (volume: 25 µL and 100 µL) with disposable tips
3. Distilled water
4. Timer with 60 min. range or higher
5. Container for the proper handling of waste and samples after use
6. Microplate washer.
7. Vortex or similar mixing tools.

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, re-centrifuge.

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, re-centrifuge.

Sample Dilution: To make 1:100 Dilution, dilute 10 ul Sample + 990 ul Sample Diluent.

Reagent Preparation:

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

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul of Standards, Controls and Diluted Sample** to the respective wells.
3. Incubate at Room Temperature for 1 hour.
3. 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.
4. Add **100 ul Anti-human-IgG:HRP Conjugate or Anti-human-IgM:HRP Conjugate** to all wells.
5. Incubate at Room Temperature for 30 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 **100 ul** of **TMB Substrate** to all wells.
8. Incubate in the dark at Room Temperature for 15 minutes.
9. Add **100 ul** of **Stop Solution**.
10. Read result with an ELISA reader at 450 nm within 5 minutes of stopping the reaction.

C a l c u l a t i o n s :

Determine the Mean Absorbance for each set of duplicate or triplicate 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 Phospholipid Screen 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 Phospholipid Screen IgG/IgM Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following case:
- If the sample absorbance value is below the first standard.

If the determination value is higher or lower than normal range, it means there is an abnormal result. The final result should be diagnosed in correlation with the clinical symptoms and other diagnostic methods.

Performance Characteristics:

Sensitivity

The clinical sensitivity of Anti Phospholipids Screen IgG assay is 92,3%.

The clinical sensitivity of Anti Phospholipids Screen IgM assay is 68,8%.

Specificity:

The clinical specificity of Anti Phospholipids Screen IgG assay is 84,6%.

The clinical specificity of Anti Phospholipids Screen IgM assay is > 99,9%.

Detection Limit

The lowest concentration that can be distinguished from zero standards is 0.03 AU/mL for IgG and 0.16 AU/mL for IgM.

Precision and reproducibility

Precision and reproducibility are evaluated by eight reply of two positive samples by two different runs with two different lots. Dispensing and washing operations were performed manually by an operator.
The results in terms of standard deviation and coefficient of variation were below:

	IgG			
Sample	**1**		**2**	
	SD	CV%	SD	CV%
Intra-assay	1.03	5.9	1.31	7.4
Inter-assay	0.26	9.2	5.25	11.7

	IgM			
Sample	**1**		**2**	
	SD	CV%	SD	CV%
Intra-assay	0.61	7.6	1.97	5.9
Inter-assay	0.15	7.1	2.98	6.6

REFERENCE VALUES
In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti Phospholipid Screen test:

	IgG (GPL AU/mL)	IgM (MPL AU/mL)
Normal	< 10	< 10
Elevated	≥ 10	≥ 10

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in 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 Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of seric Ab-Anti-Phospholipid.

LIMITATIONS OF PROCEDURE

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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Krishgen Pudgala LLP, 2023.

THANK YOU FOR USING KRISHGEN PRODUCT!

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EC REP **KinesisDx, Lyoner Strasse 14, Frankfurt, Germany**

Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

** Under CDSCO Registration, please note*

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **100 ul of Standards, Controls and Diluted Sample** to the respective wells.
3	Incubate at Room Temperature for 1 hour.
4	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.
5	Add **100 ul Anti-human-IgG:HRP Conjugate or Anti-human-IgM:HRP Conjugate** to all wells
6	Incubate at Room Temperature for 30 minutes
7	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
8	Add **100 ul** of **TMB Substrate** to all wells
9	Incubate at Room Temperature for 15 minutes
10	Add **100 ul** of **Stop Solution**
11	Read result with an ELISA reader at 450 nm within 5 minutes of stopping the reaction.

SYMBOLS KEY

MTP	Microtiter Plate (8x12 wells)
STD	Standards
NEG CNTRL	Negative Control
POS CNTRL	Positive Control
SAMP DIL	Sample Diluent
IgG HRP CONJ	Anti-human-IgG:HRP Conjugate
IgM HRP CONJ	Anti-human-IgM:HRP Conjugate
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