KRISHGEN PUDGALA LLP

Anti Cardiolipin IgM GENLISA™ ELISA

| **REF** | : KBD301M |

Ver 2.0

| **IVD** |

Enzyme Immunoassay for the Quantitative Determination of Anti Cardiolipin 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**

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** KBD301M | ∑ **96 tests** |

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Introduction:

The acronym "aPL" (anti-phospholipid antibodies) indicates improperly antibodies directed against negatively charged phospholipids like Cardiolipina (CL), Phosphatidyl serine (PS) Phosphatidyl inositol (PI) and phosphatidic acid (PA); more correctly the term anti-phospholipid antibodies indicates those antibodies directed against the complex between β2GPI and anionic phospholipids that can bind to the fifth domain of β2GPI. Among these, the Cardiolipina is the most commonly used phospholipid as an antigen for determining the aPL by ELISA method. Diagnostic laboratories measure 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 Cardiolipina 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 patient samples with suspected.

Intended Use:

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

Principle:

Anti Cardiolipin IgM GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay which is designed to quantitatively detect Anti Cardiolipin IgM present in the human serum and plasma. Anti Cardiolipin IgM test is based on the binding of antibodies on human serum or plasma directed against the antigenic complex between Cardiolipina and β2-Glycoprotein this complex is pre-coated onto microwells. Standards, Samples and Controls are pipetted into microwells Anti Cardiolipin IgM present in test sample binds to the antigen coated on the wells. And then Anti-human-IgG: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 Anti Cardiolipin IgM 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 Anti Cardiolipin 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-IgM:HRP Conjugate - 15 ml
6. (20X) Wash Buffer – 2 x 25 ml
7. Sample Diluent – 100 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. 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-IgM:HRP Conjugate** to all wells.

5. Incubate at Room Temperature for 60 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 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 Anti Cardiolipin 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 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 Cardiolipin IgM is 81.8%.

Specificity

The clinical specificity of Anti Cardiolipin IgM is > 99,9

Detection Limit

The lowest concentration of Anti Cardiolipin IgM antibodies that can be distinguished from Standards 0 is 0.12 AU/mL with a confidence limit of 95%.

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

Anti Cardiolipin IgM (AU/mL)	
Normale	< 10
Elevato	≥ 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-Cardiolipina.

Precision:
Precision and reproducibility

Precision and inter and intra assay variation are evaluated by replicates of two positive samples tested in 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 are below:

	Anti Cardiolipin IgM			
Sample	1		2	
	SD	CV%	SD	CV%
Intra-assay	3.36	10.5	2.21	10.1
Inter-assay	0.20	9.3	4.17	10.3

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

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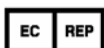
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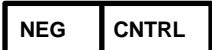
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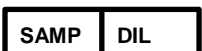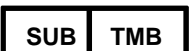
CE Marked	Europe
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 * Under CDSCO Registration, please note

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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-IgM:HRP Conjugate** to all wells.
6	Incubate at Room Temperature for 60 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 in dark 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.

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SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
STD	**Standards**
NEG CNTRL	**Negative Control**
POS CNTRL	**Positive Control**
SAMP DIL	**Sample Diluent**
IgM HRP CONJ	**Anti-human-IgM: HRP Conjugate**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
[i]	**Consult Instructions for Use**
REF	**Catalog Number**
	Expiration Date
	Storage Temperature