






GENLISA® Human Platelet Antibodies IgG/M/A, PA-IgG/M/A ELISA

REF : KBH14714

Ver 1.0


RUO

Enzyme Immunoassay for the Qualitative Determination of Human Platelet Antibodies IgG/M/A, PA-IgG/M/A in serum, plasma and other biological samples

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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 **96 tests**

Krishgen Biosystems Private Limited

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Introduction:

The GENLISA® ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antigens are used in this kit.

Intended Use:

The GENLISA® Human Platelet Antibodies IgG/M/A, PA-IgG/M/A ELISA kit is used as an analytical tool for qualitative determination of Human Platelet Antibodies IgG/M/A, PA-IgG/M/A in serum, plasma and other biological samples.

Principle:

The method employs the sandwich enzyme immunoassay technique. Human Platelet antigens are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Human Platelet Antibodies IgG/M/A, PA-IgG/M/A present in the sample are bound by the antigens. Platelet Antigen conjugated to HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human Platelet Antibodies IgG/M/A, PA-IgG/M/A in the sample. The color development is stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Human Platelet Antigen Microtitre coated plate – 8 x 12 wells
2. Positive Control - 0.5 ml
3. Negative Control - 0.5 ml
4. Platelet Antigen:HRP Conjugate - 6 ml
5. Sample Diluent - 6 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction manual

Materials to be provided by the End-User:

1. 37°C incubator
2. Standard microplate reader.
3. Precision pipettes and Disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution
6. Absorbent paper.

Storage Information:

1. All reagents should be stored at 2°C to 8°C.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25°C). Store all the components of the kit at its appropriate storage condition after use.
4. 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.
2. For Research Use Only.

Specimen Collection and Preparation:

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.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
6. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25ml** of **20X Wash Buffer** in **475 ml** of **DI water**.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human Platelet Antibodies IgG/M/A, PA-IgG/M/A. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Platelet Antibodies IgG/M/A, PA-IgG/M/A present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent.
3. Human Platelet Antibodies IgG/M/A, PA-IgG/M/A concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN₃), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Platelet Antibodies IgG/M/A, PA-IgG/M/A.
5. It is recommended that all Controls and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromise of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that Samples should be run in duplicates.
2. Add **50 ul** of **sample diluent** into blank wells.
3. **Add 50 ul Negative control and 50 ul positive control to respective control wells;**

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4. Add Sample diluent **40 ul** to testing sample well, then add testing sample **10 ul** (sample final dilute degree is 5 times). Do not add into blank control wells.
5. Incubate at 37°C for 30 minutes.
6. Aspirate and wash plate 5 times with 300 ul Wash Buffer (1x) and blot residual buffer by firmly tapping plate upside down on an absorbent paper. Wipe off 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 HRP Conjugate** to each well (Do not add into blank control well)
8. Incubate at 37°C for 30 minutes.
9. Aspirate and wash as per step no. 6 above.
10. Pipette **100 ul of TMB Substrate to each well.**
11. Incubate at 37°C for 10 minutes.
12. Add **100 ul of Stop Solution** into all wells.
13. Calibrate the plate reader with blank control well and read the plate using microwell plate reader at 450 nm.

Calculation of Results:

The Cut Off = Mean of the Negative Control + 0.15

Validity of the test:

Mean of the Positive Control ≥ 1.00 ;
Mean of the Negative Control ≤ 0.15

Interpretation of Results

Negative determinant: if the sample OD value $<$ Cut Off, the Human Platelet Antibodies IgG/M/A, PA-IgG/M/A is Negative;

Positive determinant: if the sample OD value \geq Cut Off, the Human Platelet Antibodies IgG/M/A, PA-IgG/M/A is Positive.

Safety Precautions:

- **This kit is For Research 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.
- 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.



Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A	Blank			
2A	Blank			
1B	Positive Control			
2B	Positive Control			
1C	Negative Control			
2C	Negative Control			
1D	Sample			
2D	Sample			
1E	Sample			
2E	Sample			
1F	Sample			
2F	Sample			
1G	Sample			
2G	Sample			
1H	Sample			
2H	Sample			
3A	Sample			
4A	Sample			
3B	Sample			
4B	Sample			

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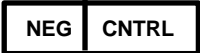









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SYMBOLS KEY

	Coated Microtiter Plate (8 x 12 wells)
	Positive Control
	Negative Control
	HRP- Conjugate
	Sample Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature