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GENLISA® Human Anti-Phenolic Glycolipid 1, ANTI-PGL1 ELISA

REF : KBH11202

Ver 1.0

RUO

Enzyme Immunoassay for the Qualitative Determination of Human Anti-Phenolic Glycolipid 1, ANTI-PGL1 in Human 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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REF KBH11202 Σ 96 tests

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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 antibodies are used in this kit.

Intended Use:

The GENLISA® Human Anti-Phenolic Glycolipid 1, ANTI-PGL1 ELISA kit is used as an analytical tool for qualitative determination of Human Anti-Phenolic Glycolipid 1, ANTI-PGL1 in Human serum, plasma and other biological samples.

Principle:

The method employs the sandwich enzyme immunoassay technique. Antibodies specific to Anti-Phenolic Glycolipid 1 are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Human Anti-Phenolic Glycolipid 1 present in the sample are bound by the antibodies. Then, HRP Conjugated to ANTI-PGL1 antibody is added 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 Anti-Phenolic Glycolipid 1, ANTI-PGL1 in the sample. The color development is stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Human ANTI-PGL1 Antibody Microtitre coated plate – 8 x 12 wells
2. Positive Control - 0.5 ml
3. Negative Control - 0.5 ml
4. ANTI-PGL1 Antibody: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. 37ºC 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 2ºC to 8ºC.
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 (18-25ºC). 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 Anti-Phenolic Glycolipid 1 Antibody. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Anti-Phenolic Glycolipid 1 Antibody 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 Anti-Phenolic Glycolipid 1 Antibody 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 (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Anti-Phenolic Glycolipid 1 Antibody.
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 compromisation 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 **50ul** of **sample diluent** into blank wells.

3. **Add 50 ul Negative control and 50 ul positive control to respective control wells**;

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 except 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 Anti-Phenolic Glycolipid 1, ANTI-PGL1 is Negative.
Positive determinant: if the sample OD value ≥ Cut Off, the Human Anti-Phenolic Glycolipid 1, ANTI-PGL1 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 2A	Blank Blank			
1B 2B	Positive Control Positive Control			
1C 2C	Negative Control Negative Control			
1D 2D	Sample Sample			
1E 2E	Sample Sample			
1F 2F	Sample Sample			
1G 2G	Sample Sample			
1H 2H	Sample Sample			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

MTP	Coated Microtiter Plate (8 x 12 wells)
POS CNTRL	Positive Control
NEG CNTRL	Negative Control
HRP CONJ	HRP- 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