GENLISA® Rotavirus IgA Antibody ELISA

REF : KBVH372

Ver 1.0

RUO

Enzmye Immunoassay for Qualitative Determination of Rotavirus IgA Antibody ELISA in serum, plasma, 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 KBVH372 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 an indirect ELISA technique.

Intended Use:

The GENLISA® Rotavirus IgA Antibody ELISA is used as an analytical tool for qualitative determination of Rotavirus IgA Antibody in serum, plasma and other biological samples.

Principle:

The method employs indirect ELISA technique. Antigens are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Rotavirus IgA Antibody present in the sample are bound by the antigens. Rotavirus IgA Antibody 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 Rotavirus IgA Antibody in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Rotavirus Antigen Coated Microtiter plate (96 wells) – 1 no.
2. Negative Control – 0.3 ml
3. Positive Control – 0.3 ml
4. Rotavirus IgA Antibody:HRP Conjugate – 10 ml
5. Sample Dilution – 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. 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. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to 2ºC - 8ºC. Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
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.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

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, re-centrifuge.
3. **Plasma**- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.
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. **Cell Lysate – for adherent cells-** Remove the culture medium and wash with PBS, normal saline or serum-free medium. Add an appropriate amount of lysis buffer and gently aspirate to ensure through cell contact. Typically cells will be lysed within 10 seconds. For suspension cells: Centrifuge to collect cells, then wash with PBS, normal saline, or serum-free medium. Add lysis buffer and aspirate to disperse cells. Gently tap with fingers to complete lysis. After complete lysis, centrifuge at 10,000 – 14,000xg for 3-5 minutes. Collect the supernatant immediately for analysis or aliquoting, then store at -20°C.

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

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

1. Before use, all components should be rewarmed at least 60 min to ensure full rewarmed to room temperature.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 25 ml 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 Rotavirus IgA Antibody. High Dose Hook Effect is due to excess of antigen for very high concentrations of Rotavirus IgA Antibody present in the sample.
3. Rotavirus IgA 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 Rotavirus IgA Antibody.
5. It is recommended that all Standards 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 Standards and Samples.

Assay Procedure:

1. All reagents and components should be restored to room temperature first. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Prepare the working solution of each component of the kit according to the method described in the previous reagent preparation.

3. Take out the required strips from the aluminium foil bag, and seal the remaining strips in a self-sealing bag and put them back in the refrigerator.

4. Set standard, sample wells on the pre-coated plate respectively, and then, record the positions.

5. Add **50 ul** of different concentration of standard product to each standard well.

6. Add **50 ul of diluted sample** to be tested to the sample well and do not add blank well.

7. Pipette **100 ul Rotavirus IgA Antibody:HRP Conjugate** to all wells.

8. Cover the plate with a sealer and incubate for 60 minutes at 37ºC.

9. Aspirate and wash plate 5 times with **Wash Buffer (1X)** 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.

10. Pipette **100 ul TMB Substrate** to all wells.

11. Cover the plate with a sealer and incubate at **37°C** for 15 minutes in dark.

12. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

13. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation 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 + 0.25

Validity of the test:

The test is valid if the following conditions are met,
Mean Absorbance of Negative Control < 0.2.
Mean Absorbance of Positive Control > 0.8.

Interpretation of Results:

Negative Results: if the OD value < CUT OFF, the sample is Negative for Rotavirus IgA Antibody.

Positive Results: if the OD value > CUT OFF, the sample Positive for Rotavirus IgA Antibody.

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.
- 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 (96 wells)
PC	Positive Control
NC	Negative Control
HRP CONJ	Conjugate Horseradish Peroxidase
SAMP DIL	Sample Dilution
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