

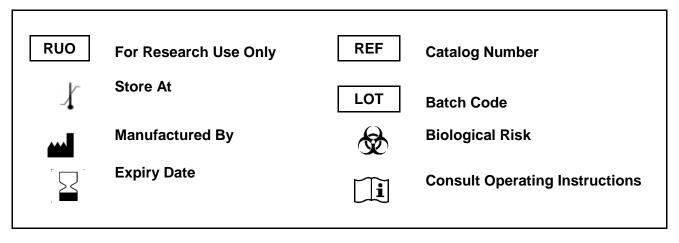
Mouse Rotavirus Antibody IgG GENLISATM ELISA

| **REF** |: KLM430

Ver4.2

RUO

Immunoassay for Qualitative Determination of Rotavirus Antibody IgG Antibodies in Mouse serum and plasma.



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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 Mouse Rotavirus Antibody IgG GENLISA™ ELISA is used as an analytical tool for qualitative determination of Mouse Rotavirus Antibody IgG antibodies in serum and plasma.

Principle:

The method employs sandwich ELISA technique. Rotavirus antigen is pre-coated onto microwells. Samples and controls are pipetted into microwells and antibodies to Rotavirus antigen present in the sample are bound by the Antibody. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific to Mouse Rotavirus Antibody IgG 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 Mouse Rotavirus IgG antibodies in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

- 1. Mouse Rotavirus Antigen Coated Microtiter plate 96 wells
- 2. Negative Control 0.5 ml
- Positive Control 0.5 ml
- 4. Rotavirus 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. 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 room temperature (18-25°C). 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.



Specimen Collection and Handling:

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

- 1. The kit cannot test samples which contain NaN₃, because NaN₃ inhibits HRP activity.
- 2. 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.
- 3. **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.
- 4. **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.

Test Sample Preparation: Add 10 ul of sample and to this add 40 ul of sample diluent, mix well with gently shaking. Samples should be loaded onto the bottom without touching the well wall.

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

- 1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
- 2. To make Wash Buffer (1X); dilute 25 ml of (20X) Wash Buffer in 475 ml of Dl water.

Procedural Notes:

- 1. Read all the instructions thoroughly before performing the test.
- 2. Allow all reagents to reach Room Temperature before beginning and reconstitute or dilute the required reagents.
- 3. The kit cannot be used for samples with NaN3 which can inhibit the activity of HRP.
- 4. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
- 5. All Controls and Samples should be assayed at least in duplicates.
- 6. The assay has been optimized to be used with the protocol mentioned. Any deviation from the same may invalidate the results.

Assay Procedure:

- 1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. All steps must be performed at 37°C.
- 2. Pipette 50 ul of Positive Control, Negative Controls and Diluted Samples into the respective wells.
- 3. Cover the plate and incubate for 30 minutes at 37°C.
- 4. 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.
- 5. Add 50 ul of Rotavirus Antigen: HRP Conjugate into each well except the blank control well.
- 6. Cover the plate and incubate for 30 minutes at 37°C.

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- 7. Aspirate and wash as per Step (4) above.
- 8. Add 100 ul of TMB Substrate in each well.
- 9. Incubate the plate at 37°C for 15 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
- 10. Pipette out 100 ul of Stop Solution. Wells should turn from blue to yellow in color.
- 11. 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 + 0.15

Validity of the test:

The test is valid if the following conditions are met, Mean Absorbance of Negative Control ≤ 0.10 Mean Absorbance of Positive Control ≥ 1.00

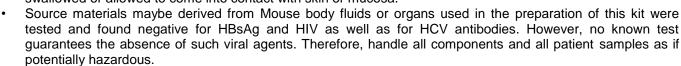
Interpretation of Results:

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

Positive Results: if the OD value ≥CUT OFF, the sample Positive for Rotavirus Antibody IgG.

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.
- 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.





- 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.







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SYMBOLS KEY

МТР	Coated Microtiter Plate (96 wells)
PC	Positive Control
NC	Negative Control
HRP CONJ	Rotavirus Antigen:HRP Conjugate
SAMP DIL	Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
[]i	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
1	Storage Temperature