GENLISA® Rat Hanta Virus Antibody IgG ELISA

REF : KLR220

Ver 1.4

RUO

Enzyme Immunoassay for Qualitative screening of Antibodies against Rat Hanta Virus Antibody IgG in Rat sera

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 KLR220 Σ 1 x 96 tests

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Introduction:

Hantavirus is classified as a negative sense RNA virus belonging to bunyaviridae family. This virus is transmitted to humans most commonly through rats and mice bites. Common disease caused due to the infection of hanta viruses are Korean fever (HFRS) and Hanta Pulomary Syndrome (HPS). Since rats and mice are rarely or not infected by the Hantavirus, they are just carriers of the virus.

Intended Use:

The GENLISA® Rat Hanta Virus Antibody IgG ELISA is used as an analytical tool for qualitative laboratory screening of presence or absence of antibodies against Hanta Virus Antibody IgG in the serum of the rat.

Principle:

The method employs Indirect enzyme immunoassay technique. Hanta Virus (LCMV) antigen and control antigen is pre- coated onto microwells. Samples and Controls are pipetted into microwells and Rat Hanta Virus antibodies present in the sample are bound by the antigen. Then, Anti-Rat IgG:HRP (horseradish peroxidase)-Conjugate 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 Rat Hanta Virus antibodies present in the sample. The color development is stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtitre coated plate (96 wells) - 1 no
   1 holder containing 6 Positive Viral Antigen coated strips ringed in black color and 6 Negative Viral Antigen coated strips ringed in red color.
2. Positive Control – 0.5 ml
3. Negative Control – 0.5 ml
4. Calibrator – 50 ul
5. Anti-Rat IgG:HRP Conjugate – 5.5 ml
6. (20X) Wash Buffer – 25 ml
7. Dilution buffer - 30 ml
8. TMB Substrate – 12 ml
9. Stop Solution – 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Timer.
6. Absorbent paper

Handling/Storage:

1. Reconstitute or dilute only the specific reagents mentioned in the reagent preparation section, when ready to run the assay.
2. Store all kit components at 4ºC to 8ºC when not in use and do not expose them to temperatures greater than 37ºC or less than 2ºC
3. Do not use kit components after the expiration date.
4. Do not repeatedly freeze/thaw the reagents as loss of activity may result.
5. Before using, bring all components to Room Temperature (18-25ºC). Upon assay completion return all components to appropriate storage conditions.
6. Keep the plates sealed in the pouch in the refrigerator when not in use.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. Handle Stop Solution carefully. Obtain medical attention in case of accidental ingestion of kit components.
3. Avoid assay of samples containing Sodium azide as it is hazardous.

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

1. 1X Wash Buffer:
   Dilution: To make **1X Wash Buffer**, add **25ml** of **20X Wash Buffer** to **475ml** of **DI water**. This is the working solution.
2. Dilute the calibrator serum 1:51 in Dilution buffer. For example: add 5 ul of calibrator serum to 250 ul of Dilution Buffer. If not assayed immediately, diluted serum should be stored at -20°C or below.

Specimen Collection and Preparation:

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

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles.

Dilute the serum 1:51 in Dilution buffer. For example: add 5 ul of serum sample to 250 ul of Dilution buffer. If not assayed immediately, diluted samples should be stored at -20°C or below.

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. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
4. All Controls and Samples should be assayed at least in duplicates.
5. The assay has been optimized to be used with the protocol mentioned. Any deviation from the same may invalidate the results.

Assay Procedure:

1. Bring all reagents to Room Temperature prior to use. It is strongly recommended that all **Controls and Samples** should be run in duplicates or triplicates.

2. The holder contains 12 strips alternating between Negative Viral Antigen ringed in red color and Positive Viral Antigen ringed in black color. Each control, calibrator, and specimen requires two wells: a Negative Viral Antigen coated well and Positive Viral Antigen coated well.

3. Add 50 ul of **Negative Control, Positive Control** and diluted **Sample** to the appropriate wells (Controls are ready to use and do not require any dilution step).

4. Mix the contents in the wells by moving the plate in rapid circular motion, see to it that the contents do not spill. Incubate at Room Temperature for 30 minutes.

5. Aspirate and wash plate 5 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on 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 or alternatively a microtiter plate or strip washer may be used.

6. Add **50 ul** of **Anti-Rat IgG:HRP Conjugate** to the wells.

7. Incubate at **Room Temperature for 30 minutes**.

8. Wash the plate as per the instruction given in step 5.

9. Add **100 ul** of **TMB Substrate** solution into each well of the plate. Incubate for 10 minutes.

10. Add **100 ul** of **Stop Solution** to each well.

11. Measure the optical density of the wells on a plate reader at 450 nm within 15 minutes.

Interpretation of the Results:

1. It is recommended that each laboratory establish their own criteria for performance of these Research Reagents.
2. In our quality control testing, we use the following criteria:
3. The Positive Control Serum, after subtracting the absorbance in the Negative Control Antigen well, should produce a net absorbance on the Positive Viral Antigen of ≥1.00 at 450 nm.
4. A sample may be considered Positive if the index is **1.0 or greater,** if the index is **less than 1.0** then the test is considerd as negative.

Formula:

Index value = $OD_{sample/calibrator}$ / $OD_{calibrator}$

$OD_{sample/calibrator}$ = OD of the sampel / control.
$OD_{calibrator}$ = OD of calibrator.

Calculation example:

	Positive Antigen well OD	Negative Control Antigen well OD	Differential OD
Negative control	0.12	0.04	0.08
Positive control	1.82	0.03	1.79
Calibrator	0.38	0.10	0.28
Specimen 1	1.10	0.19	0.91
Specimen 2	0.25	0.02	0.23

Index value = $OD_{sample/calibrator}$ / $OD_{calibrator}$

Differential OD of calibrator	0.28		
Index value of Negative Control	0.08 / 0.28	0.29 index	Valid
Index value of Positive control	1.79 / 0.28	6.39 index	Valid
Index value of specimen 1	0.91 / 0.28	3.25 index	Positive
Index value of specimen 2	0.23 / 0.28	0.82 index	Negative

Expected Values:

The normal value is Negative. Studies have shown that antibodies may take up to 21 days to appear after exposure. Negative specimen results should be reviewed in relation to a possible exposure date. All Positive specimen results should be confirmed by an alternate method.

Precautions:

1. Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.
2. Substrate is light and heat sensitive hence do not expose it to direct sunlight while pipetting or incubating.
3. Samples and kit reagents after use should be disposed off observing appropriate regulations.
4. If necessary it is recommended that the results should be confirmed by an alternative method.
5. Do not dilute or adulterate test reagents or use samples not called for in the test procedure.

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PLATE MAP

Assay Name:_____ Date:_____

Lot No: _____ Plate:_____

	Positive (Black)	Negative (Red)	Positive (Black)	Negative (Red)	Positive (Black)	Negative (Red)	Positive (Black)	Negative (Red)	Positive (Black)	Negative (Red)	Positive (Black)	Negative (Red)
	1	2	3	4	5	6	7	8	9	10	11	12
A	Reactive Control		Sample 6		Sample 14		Sample 22		Sample 30		Sample 38	
B	Negative Control		Sample 7		Sample 15		Sample 23		Sample 31		Sample 39	
C	Calibrator		Sample 8		Sample 16		Sample 24		Sample 32		Sample 40	
D	Sample 1		Sample 9		Sample 17		Sample 25		Sample 33		Sample 41	
E	Sample 2		Sample 10		Sample 18		Sample 26		Sample 34		Sample 42	
F	Sample 3		Sample 11		Sample 19		Sample 27		Sample 35		Sample 43	
G	Sample 4		Sample 12		Sample 20		Sample 28		Sample 36		Sample 44	
H	Sample 5		Sample 13		Sample 21		Sample 29		Sample 37		Sample 45	