GENLISA™ Mouse Hepatitis Virus (MHV) ELISA

Cat. No: KLM230

Ver1.2

Enzyme Immunoassay for qualitative screening of antibodies against Mouse Hepatitis Virus in mouse 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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Introduction:

Mouse hepatitis virus belongs to the family coronaviridae. It causes and epidemic murine illness with high mortality. It is ssRNA virus which is mostly transmitted through respiratory aerosols. Although MHV infection may be asymptomatic, it is highly contagious.

Intended Use:

The Mouse Hepatitis Virus GENLISA™ ELISA is used as an analytical tool for qualitative laboratory screening of presence or absence of antibodies against mouse hepatitis virus in the serum of mouse.

Principle:

The method employs the sandwich enzyme immunoassay technique. Hepatitis specific antigen and control antigen is pre-coated onto microwells in alternating columns. Samples, Calibrator and Controls are pipetted into microwells and Mouse Hepatitis Virus antibodies present in the sample are bound by the antigen. Then, Anti-Mouse 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 Mouse Hepatitis 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) – 2 nos.
   2 holders containing each of 12 positive and 12 negative coated strips
2. Positive Control - 1 ml
3. Negative Control - 1 ml
4. Dilution Buffer – 2 x 30 ml
5. Wash Buffer (20X) – 3 x 25 ml
6. Enzyme Conjugate - 11 ml
7. TMB Substrate - 11 ml
8. Stop Solution - 11 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 405 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 4^0C to 8^0C when not in use and do not expose them to temperatures greater than 37^0C or less than 2^0C
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. Calibrator:
   Dilute it to **1:51** in Dilution Buffer, add **5 ul** of **Calibrator** to **250 ul** of **dilution buffer.**

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 Calibrator, 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. Fit the strip holder with the required number of pre-coated Positive Viral Antigen and Negative Viral Antigen. Mark the appropriate strips with a (+) or (-). Allow one well for the Negative Control and one well for Positive Control Sera.

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.

5. Aspirate and wash plate 5 times with **Wash Buffer (1X)** 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-Mouse 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 **50 ul** of **TMB Substrate** solution into each well of the plate. Incubate for 10 minutes.

10. Add **50 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 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 by the following criteria: Determine the difference (Δ) 450 nm on the Negative Control Antigen well. This difference (Δ) should be greater than or equal to 1.000 for a sample to be considered Positive.
5. A Value of **1.0 or greater** (after subtracting the absorbance of the Negative Control antigen well) is considered **Positive**.
6. A Value of **less than 1.0** (after subtracting the absorbance of the Negative Control antigen well) is considered **Negative**.

Expected Values:

The normal value is Negative. Studies have shown that antibodies may take up to 21 days to appear after exposure; therefore, 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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