HEV IgM GENLISA™ ELISA

REF : KBD901

Ver 1.0

IVD

Enzyme Immunoassay for Qualitative Determination of Hepatitis E Virus IgM in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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REF KBD901 Σ 96 tests

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Introduction:

Hepatitis E virus (HEV), an RNA virus of the *Hepeviridae* family, is an enterically transmitted, self-limiting, acute, viral hepatitis. Transmission is through large-scale waterborne epidemics and few epidemics have spread through person-to-person contact or fecally contaminated food. Vertical transmission of HEV from infected mother to fetus causes high fetal and perinatal mortality. Other means of transmission are such as zoonotic transmission, which can fluctuate depending upon the region and strain of the virus.

Intended Use:

The HEV IgM GENLISA™ ELISA is intended for the qualitative determination of Hepatitis E Virus IgM in human serum and plasma.

Principle:

HEV IgM GENLISA™ ELISA method employs sandwich enzyme linked immunosorbent assay (ELISA) technique. Purified anti-human IgM are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Hepatitis E Virus IgM present in the sample are bound by the antibodies. Enzyme labeled antigen is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (A and B) is added to microwells and color develops proportionally to the amount of Hepatitis E Virus IgM present in sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. Negative Control - 1 ml
3. Positive Control - 1 ml
4. Enzyme Conjugate - 11 ml
5. (40X) Wash Buffer - 20 ml
6. Sample Diluent - 11 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. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. 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. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

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.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 2.5 ml of Wash Buffer (40X) to 97.5 ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Sample Diluent** to the sample wells.
3. Add **10 ul Sample** to the respective sample wells. Mix gently.
4. Dispense **50 ul Positive Control** and **50 ul Negative Control** to the respective Control wells.
5. Shake gently for 30 seconds to mix well. Incubate at 37ºC for 20 minutes.
6. Aspirate and wash plate 5 times with **(1X) Wash Buffer** 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. All the washes should be performed similarly.
7. Add **100 ul** of **Enzyme Conjugate** to each well except the blank well.
8. Incubate at 37ºC for 20 minutes.
9. Repeat the Wash step 6.
10. Add **100 ul** of **TMB Substrate** to all wells.
11. Incubate at 37ºC for 10 minutes.
12. Add **100 ul** of **Stop Solution**.
13. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation 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 + 0.10

Note: Incase negative control OD value is <0.05, Cut-Off Value is calculated as 0.05 + 0.10. Incase negative control OD value >0.05, the Cut-Off Value is calculated as Actual OD_{value} of NC + 0.10

Positive Results: **OD value ≥ CO**
Specimens giving an absorbance equal to or greater than the CO are considered initially reactive, which indicates that Anti-HEV antibody IgM has probably been detected using the ELISA.

All initially reactive specimens should be retested in duplicates using the Anti-HEV antibody IgM ELISA before the final assay results interpretation. Repeatedly reactive specimens may be considered positive for Anti-HEV antibodies with the Anti-HEV antibody IgM ELISA.

Negative Results: **OD value < CO**
Specimens giving absorbance less than the CO are negative for the assay, which indicates that no Anti-HEV antibody has been detected with the Anti-HEV antibody IgM ELISA.

Cut Off Value	OD_{mean} of Negative Control + 0.10
Positive	>= CO
Negative	< CO

Criteria of Validation:

Anti-HEV antibody IgM results are considered to be valid, if
OD of Positive Control > Cut-Off Value

OD = Optical Density / Absorbance at 450nm

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:

Sensitivity:
Limit of Detection: When detecting anti-ovary antibody limits, laboratory quality control positive samples diluted till 1:8 with the Anti-HEV antibody IgM ELISA kit should be in the positive.

Specificity:
The recombinant antigen used in the kit is specific for HEV antibody.

Precision:
Intra-Assay: CV% ≤15%.
Inter-Assay: CV% ≤20%

Safety Precautions:

- **This kit is For In-vitro Diagnostic 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.

- 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.

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Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **100 ul Sample Diluent** to the **sample wells**.
3	Add **10 ul Sample** to the **respective sample wells**. Mix gently.
4	Dispense **50 ul Negative Control** and **50 ul Positive Control** to the control wells respectively.
5	Shake gently for 30 seconds to mix well. **Incubate** at **37ºC** for **20 minutes**.
6	Aspirate and **wash plate 5 times** with **(1X) Wash Buffer** 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. All the washes should be performed similarly.
7	Add **100 ul** of **Enzyme Conjugate** to each well.
8	Seal the plate and **incubate** at **37ºC** for **20 minutes**.
9	Repeat the Wash step 6.
10	Add **100 ul** of **TMB Substrate** to all wells.
11	**Incubate** at **37ºC** for **10 minutes**.
12	Add **100 ul** of **Stop Solution**. **Read result with an ELISA reader at 450 nm** within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
CTRL	**Controls**
CONJ	**Enzyme Conjugate**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**