HAV IgM GENLISA™ ELISA

REF : KBD905

Ver 1.0

IVD

Enzyme Immunoassay for the Qualitative Determination of Hepatitis A IgM antibody 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**

REF **KBD905** **Σ 96 tests**

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Introduction:

Hepatitis A belongs to the *Hepatovirus* genus of the family *Picornaviridae*. The genome of HAV is a positive-strand RNA. It is one of the most common infectious etiologies of acute hepatitis worldwide. The virus is known to be transmitted fecal-orally *via* contaminated food or water, or through close contact with an infected person resulting in symptoms ranging from asymptomatic infection to fulminant hepatitis.

Intended Use:

The HAV IgM GENLISA™ ELISA is intended for the qualitative determination of HAV in human serum and plasma.

Principle:

HAV 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 HAV IgM antibody present in the sample is bound by the antibodies. Enzyme labeled antigen is 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 is added to microwells and color develops proportionally to the amount of HAV IgM antibody 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. Positive Control - 1 ml
3. Negative Control - 1 ml
4. Enzyme Conjugate - 6.5 ml
5. (40x) Wash Buffer - 20 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 12 ml
8. 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 minutes 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 minutes at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Reagent Preparation:

1. (1x) Wash Buffer Dilution: To make (1x) Wash Buffer, add 2.5 ml of (40x) Wash Buffer 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 **50 ul Sample and Control** to the well.
3. Incubate for 30 minutes at 37°C.
4. 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.
5. Add **50 ul Enzyme Conjugate** to sample wells. Mix well.
6. Incubate for 30 minutes at 37°C.
7. Repeat the Wash step 6.
8. Add **100 ul** of **TMB Substrate** into each well except blank well.
9. Incubate at 37°C for 10 minutes.
10. Add **100 ul** of **Stop Solution**.
11. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance (net of Blank) 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 x 2.1

Note: Incase Negative Control OD Value is <0.05, the Cut-Off Value is calculated as OD_{mean} of NC (to be taken as) 0.05 x 2.1.
Incase Negative Control OD Value is >0.05, the Cut-Off Value is calculated as OD_{mean} of NC (actual value) x 2.1

Positive Results: **OD value ≥ CO**
Specimens giving an absorbance equal to or greater than the CO are considered initially reactive, which indicates that HAV IgM antibody has probably been detected using the HAV IgM antibody ELISA.
All initially reactive specimens should be retested in duplicates using the HAV IgM antibody ELISA before the final assay results interpretation. Repeatedly reactive specimens may be considered positive for HAV IgM antibody with the HAV IgM antibody ELISA.

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

Cut Off Value	OD_{mean} of Negative Control x 2.1
Positive	>= CO
Negative	< CO

Criteria of Validation:

HAV IgM antibody ELISA results are considered to be valid, if
OD of Positive Control > Cut-Off Value, and
OD of Positive Control >= 0.8

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.

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-HAV antibody limits, laboratory quality control positive samples diluted till 1:8 with the HAV IgM antibody ELISA kit should be in the positive.

Specificity:
The recombinant antigen used in the kit is specific for HAV 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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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 **50 ul Sample and Control to respective wells. Incubate** at **37ºC** for **30 minutes**
3	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.
4	Add **50 ul** of **Conjugate** to each well except the blank well.
5	**Incubate** at **37°C** for **30 minutes**
6	Repeat the Wash step 6
7	Add **100 ul** of **TMB Substrate** into each well except blank well.
8	**Incubate** at **37°C** for **10 minutes**.
9	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 (8 x 12 wells)**
CTRL	**Controls**
CONJ	**Enzyme Conjugate**
40x WASH BUF	**(40x) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**