KRISHGEN PUDGALA
PARTNER FOR PRECISION DIAGNOSTICS

HAV IgM GENLISA® ELISA

REF : KBD905

Ver 1.3

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**

For *In-vitro Diagnostic Use only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN Pudgala LLP is strictly prohibited.*

REF KBD905 Σ 96 tests

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Village Dapode, Bhiwandi 421302.

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:

The 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 Conjugate 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. (20X) Wash Buffer - 25 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-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. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 25 ml of Wash Buffer (20X) to 475 ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **50 ul Sample, Positive and Negative Controls** to the respective wells (Do not add in the blank well).
3. Shake gently for 30 seconds to mix well. Incubate at 37ºC for 30 minutes.
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 each well except blank well.
6. Incubate for 30 minutes at 37°C.
7. Repeat the Wash step 4.
8. Add **100 ul** of **TMB Substrate** into each well, except blank well.
9. Incubate at 37ºC for 10 minutes.
10. Add **100 ul** of **Stop Solution** into each well except blank well.
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 Anti-HAV antibody IgM has probably been detected using the ELISA.

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

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

Criteria of Validation:

Anti-HAV antibody IgM results are considered to be valid, if

Mean Absorbance of Negative Control ≤ 0.1
Mean Absorbance of Positive Control ≥ 0.8

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: The positive endpoint for the serial diluted reference in the national quality control samples should not be less than 1:16.

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 Invitro 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.

LIMITED WARRANTY

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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This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Pudgala LLP, 2026

THANK YOU FOR USING A KRISHGEN PRODUCT!

Krishgen Pudgala LLP

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Regulatory Status:

CE Marked	Euro
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, Positive and Negative Controls** to the respective well (Do not add in the blank well).
3	Shake gently for 30 seconds to mix well. Incubate at 37ºC for 30 minutes.
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 each well except blank well
6	Incubate for 30 minutes at 37°C.
7	Repeat the Wash step 4.
8	Add **100 ul** of **TMB Substrate** into each well, except blank well
9	Incubate at 37ºC for 10 minutes.
10	Add **100 ul** of **Stop Solution** into each well except blank well.
11	Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	**Microtiter Plate (96 wells)**
POS CNTRL	**Positive Control**
NEG CNTRL	**Negative Control**
ENZY CONJ	**Enzyme Conjugate**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**