BIOGENIX
INC. PVT. LTD.

Document No. BIPL-F-IFU-01, Issue No. 01, Revision No. 00, Effective Date 03/01/2019,

Hepatitis A Virus (HAV) IgM Antibody Enzyme Immunoassay Test Kit
（ELISA）New version

INTENDED USE

This kit is a qualitative detection of human serum/plasma of hepatitis A virus IgM. The kit is suitable for clinical screening and diagnosis of hepatitis A virus infection in serum/plasma.

SUMMARY AND EXPLANATION

Hepatitis A virus (HAV) is a kind of RNA virus, which belongs to the family of Picornavirus. HAV showed a spherical particles shape with 27 nm in diameter, icosahedral symmetry consisted of 32 shell particles, containing linear single-strand RNA. Hepatitis A is a kind of intestinal infectious disease caused by hepatitis A virus (HAV) with a worldwide distribution. Children and adolescents are most likely to be infected, and the peak incidence is in winter and spring. Hepatitis A is the most common type of acute viral hepatitis, mainly spread by fecal-oral route. The incubation period of hepatitis A virus is 15 to 45 days, HAV-IgM antibody can be detected in serum/plasma a short time later after infected, continue to rise very rapidly, peaking in about 2 weeks, decreasing gradually, disappear in 8 weeks. While HAV-IgG antibody appear later than IgM and will persistent for a long time. Detection of HAV- IgM antibody can diagnose HAV infection in early stage for its simple, fast, and high specificity.

PRINCIPLE OF THE TEST

This kit uses capture ELISA principle to detect HAV IgM. Purified anti-human IgM antibody is pre-coated on the microplate, the HAV IgM in sample will combine with HAV antigen first, then combine with enzyme-labeled anti-HAV HAV antigen complex, and shows blue color in the microplate. This kit is used for the specific detection of HAV IgM antibody in human serum/plasma.

COMPONENTS

Materials provided with the kit:

	96T		48T	
Coated Microtiter plate	1 bag	12*8	1 bag	12*4
HRP Conjugate	1 vial	6.5 mL	1 vial	3.5 mL
Wash Buffer Concentrate (40*)	1 vial	20 mL	1 vial	10 mL
Substrate A	1 vial	7 mL	1 vial	3.5 mL
Substrate B	1 vial	7 mL	1 vial	3.5 mL
Stop Solution	1 vial	6 mL	1 vial	3 mL
Negative control	1 vial	1 mL	1 vial	1 mL
Positive Control	1 vial	1 mL	1 vial	1 mL
Closure plate membrane	3 sheet		3 sheet	

Note: different batches of reagent kit, and different component can not be exchanged for use. Once open, stable for 3 months at 2-8°C.

SPECIMEN COLLECTION AND PREPARATION

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum/plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum must be separated from the clot as early as possible to avoid haemolysis of the RBC. Care should be taken to ensure that the serum/plasma specimens are clear and not contaminated by microorganisms.

2. ***Highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay. ***Do not heat inactivate specimens***. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. HAV ELISA is intended ONLY for testing of individual serum/plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

TEST PROCEDURE

1. All reagents should be allowed to reach room temperature for 15 minutes before use.

2. Add 50μL samples in the corresponding hole or positive and negative controls (Do not add in the blank hole). The sample corresponding to the number of micro plate, each plate should be provided with negative control 2 holes, positive control 1 hole and blank control 1 hole. (If detect with dual wavelength detection, setting no blank control hole is allowed).

Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control to avoid cross-contamination.

3. Shake gently to mix for 30 s. Incubate at 37 ℃ for 30 minutes with the sealing plate membrane sealing the plate

4. Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use. At the end of the incubation, remove and discard the plate cover. Take out, add wash buffer to each well for 20 seconds. Repeat 5 times. After the final washing cycle, turn the plate over onto blotting paper or clean towel, and tap it to remove any remainders.

5. Respectively adding HRP Conjugate 50μL (Do not add in the blank hole)

6. Incubate at 37 ℃ for 30 minutes with the sealing plate membrane sealing plate. Repeat the wash step for 5 times as in step 4.

7.Add Substrate A and Substrate B 1 drop (50μL) (Do not add in the blank hole). Incubate at 37 ℃ for 10

minutes with the sealing plate membrane sealing the plate.

8. Add 50μL Stop Solution to each well (Do not add in the blank hole). Mix gently by shaking, read the absorbance within 10 minutes after stopping the reaction. Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Set no blank holes is allowed if use dual wavelength to detect. Calculate the Cut-off value and evaluate the results.

INTERPRETATION OF RESULTS

Colorimetry: Read O.D at 450nm with a microplate reader.

Mean negative control O.D≤0.1and positive control O.D≥0.8, the test is valid, otherwise the test is invalid.

Cut off=Mean negative control A x2.1(Calculated by 0.05 when Mean negative control O.D. is ＜ 0.05, calculated by actual value when Mean negative control O.D. is ＞0.05)

Positive Results: Sample O.D ≥ Cut-off O.D.

Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that HAV IgM has probably been detected using HAV ELISA. All initially reactive specimens should be retested in duplicates using HAV ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for HAV IgM with HAV ELISA.

Negative Results: Sample O.D < Cut-off O.D.

Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HAV IgM has been detected with HAV ELISA, therefore the patient is probably not infected with HAV and the blood unit do not contain HAV IgM.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

LIMITATIONS OF PROCEDURE

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. The reagent is a qualitative reagent, and can not be used as a quantitative reagent.
3. This reagent is only used for the detection of human serum/plasma samples.

PRECAUTIONS

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. Allow the reagents and specimens to reach room temperature before use. Shake reagent gently before use.
4. Concentrated washing liquid will produce crystal at room temperature, should be diluted completely before use.
5. Please put the unused plate back into the bag, and store at 2～8℃.
6. Operate strictly according to the instruction, control of reaction time and temperature strictly.
7. Never reuse microplate sealing membrane. If the external of the microplate contact with water when warm bath, results will be better.
8. Use sufficient volume of washing liquid in the washing steps. Fail to do so may cause color deepening.
9. Negative results of reagent do not rule out the possibility of HAV infection. Positive results must be combined with clinical information for analysis.
10. All the reagents were treated by inactivation, but still should be regarded as potentially infectious. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

STORAGE & VALIDITY

1. Store at 2-8℃.DO NOT FREEZE.
2. Once open, stable for 3 months at 2-8°C. Other liquid components have the same validity period with the reagent box.

REFERENCES

Chinese Pharmacopoeia
China Biological Products Procedures

	Keep in Dark Place		Keep Dry
	Do Not Reuse		Temperature Limitation
	Consult Instruction for Use		In Vitro Diagnostic Medical Product
	Batch Code		Contains Sufficient for <n> Tests
	Manufacturer		Date of Manufacture
	This side up		Fragile

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