Human HAV IgM antibody ELISA Kit

Human HAV IgM antibody ELISA Kit is an Enzyme Immunoassay kit for the determination of Human HAV IgM antibody in serum and plasma (EDTA, heparin, citrate).

Catalog number: ARG82304

Package: 96 wells

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INTRODUCTION

Hepatitis A is an infectious disease of the liver caused by Hepatovirus A (HAV); it is a type of viral hepatitis. Many cases have few or no symptoms, especially in the young. The time between infection and symptoms, in those who develop them, is 2–6 weeks. When symptoms occur, they typically last 8 weeks and may include nausea, vomiting, diarrhea, jaundice, fever, and abdominal pain. Around 10-15% of people experience a recurrence of symptoms during the 6 months after the initial infection. Acute liver failure may rarely occur, with this being more common in the elderly.

It is usually spread by eating food or drinking water contaminated with infected feces. Undercooked or raw shellfish are relatively common sources. It may also be spread through close contact with an infectious person. While children often do not have symptoms when infected, they are still able to infect others. After a single infection, a person is immune for the rest of his or her life. Diagnosis requires blood testing, as the symptoms are similar to those of a number of other diseases. It is one of five known hepatitis viruses: A, B, C, D, and E. [Provide by Wikipedia: HAV]

PRINCIPLE OF THE ASSAY

This assay employs the enzyme immunoassay technique. The solid phase of the microplate is made of polystyrene wells coated with Anti-Human IgM. When a serum or plasma sample containing Anti-HAV IgM is added to the wells coated with Anti-Human IgM antibodies and incubated, the IgM antibodies in

the sample bind to the wells with anti-Human IgM. After adding the solution containing Ag HAV and a conjugated solution of Anti-HAV·Peroxidase and incubating, an anti-HAV IgM immunocomplex is formed – (Anti-Human IgM)-(Anti-HAV IgM) – (Ag HAV) – (Anti-HAV·Peroxidase). After washing to remove the unbound material, a substrate solution with TMB is added to the wells and incubated. It develops a color, proportional to the amount of Anti-HAV IgM. Then the Stop Solution is added to the wells to stop the reaction. The optical density of developed color is read with a plate reader at O.D. 450 nm with a reference wavelength within 620 to 690 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Do not freeze. Use the kit before expiration date.

Component	Quantity	Storage information
Anti-Human IgM Coated Microplate	96 wells	4°C
Anti-HAV·Peroxidase Solution	8 mL (ready to use)	4°C
Anti-HAV IgM Positive Control	2.5 mL (ready to use)	4°C
Anti-HAV IgM Negative Control	2.5 mL (ready to use)	4°C
Specimen Diluent Buffer	12 mL (ready to use)	4°C
HAV Solution	8 mL (ready to use)	4°C
20X Wash Buffer	58 mL	4°C

TMB Substrate A	12 mL	4°C (protect from light)
TMB Substrate B	12 mL	4°C (protect from light)
Stop Solution	12 mL (ready to use)	4°C

Note:

- Adhesive Slips / Absorbent Pads / Black Cover provide as needed.
- All component are stable for up 1 month once open.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620-690 nm as optional reference wave length)
- Centrifuge and centrifuge tube
- Incubator (37°C)
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The positive control, negative control, HAV Solution, Anti-HAV·Peroxidase Solution and specimens should be regarded as potential hazards to health. They should be used and discarded according to the user's laboratory

safety procedures.

- Return any unused microplate strips to the plate pouch with desiccant.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-30°C).
- The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C.

Note:

- Whole blood specimen should be separated as soon as possible in order to avoid hemolysis.
- Specimens must be stored at 4°C and avoided heat inactivation to minimize deterioration.
- For long term storage, specimens should be frozen below -20°C. Storage in self-defrosting freezers is not recommended.
- Avoid multiple freeze-thaw procedures.
- The specimen must not contain any AZIDE compounds which can inhibit the peroxidase activity.
- Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 mL of 20X Wash Buffer into 475 mL of distilled water to a final volume of 500 mL)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-30°C) before use. Standards and samples should be assayed in duplicates.

1. Make 1:200 dilution of each specimen. Prepare for each specimen a tube for dilution. Add 1 mL of Specimen Diluent Buffer and 5 µL of each specimen to each tube respectively and shake to mix.
   Note: Do not dilute the controls.
2. Reserve 1 wells for blanks. Add 100 µL of Negative Control to each three wells, 100 µL of Positive Control to each two wells, and 100 µL of Specimen Diluent Buffer to each other of the reaction wells for test sample.
3. Add 5 µL of each diluted specimen to each well containing Specimen Diluent Buffer, respectively.
4. Gently tap the plate. Cover the plate and incubate for 1 hour at 37°C.
5. Remove plate cover. Aspirate each well and wash, repeating the process 7 times for a total 8 washes. Wash by filling each well with 1X Wash Buffer (350 µL) using a squirt bottle, manifold dispenser. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

6. Add 50 µL of HAV Solution to each reaction well except the blank well.
7. Add 50 µL of Anti-HAV·Peroxidase Solution to each well except the blank well.
8. Gently tap the plate. Cover the plate and incubate for 1 hour at 37°C.
9. Aspirate each well and wash as step 5.
10. Add 50 µL of TMB Substrate A first, then add 50 µL of TMB Substrate B to each well including the blank well. Mix well gently.
11. Cover the plate and incubate for 30 minutes at room temperature in the dark.
12. Immediately Add 100 µL of Stop Solution to each well including the blank well. The color of the solution should change from blue to yellow.
13. Read the absorbance at O.D. 450 nm. (with 620-690 nm reference wavelength)

    Note: absorbance value of blank well must be less than 0.100.

CALCULATION OF RESULTS

1. Calculation of the NCx (Mean Absorbance of Negative Control).

   Example:

Sample No.	Absorbance
1	0.080
2	0.085
3	0.079

   NCx = (0.080 + 0.085 + 0.079) / 3 = 0.081 (NCx must be ≤ 0.2, otherwise, the test run is invalid).

2. Calculation of the PCx (Mean Absorbance of Positive Control).

   Example:

Sample No.	Absorbance
1	1.223
2	1.205

   PCx = (1.223 + 1.205) / 2 = 1.214 (**PCx must be ≥ 0.5, otherwise, the test run is invalid**).

3. Calculation of the P – N value

   Example:

   P – N = PCx – NCx = 1.214 – 0.081 = 1.133 (**P-N value must be ≥ 0.3, otherwise, the test run is invalid**).

4. Calculation of the Cutoff Value

   Cutoff Value = NCx + (PCx / 4)

   Example: Cutoff Value =0.081 + (1.214 / 4) = 0.385

5. Calculation of the Retest Range

   Retest Range = Cutoff Value ± 10%

   Example: Cutoff Value = 0.385,

   Retest Range = (0.385 – 0.039) to (0.385 + 0.039) = 0.346 to 0.424

INTERPRETATION OF RESULT

- Specimen with absorbance values LOWER than the cutoff value are considered non-reactive for Anti-HAV IgM.
- Specimen with absorbance values GREATER than or EQUAL TO the cutoff value are considered reactive for Anti-HAV IgM.
- If the data is within Retest Range, the test must be repeated in duplicate and interpreted as above. If the retested absorbance still within the retest range, it is suggested to test follow-up-samples.

QUALITY ASSURANCE

Sensitivity

61.7 GBU/mL