Human HAV antibody ELISA Kit

Enzyme Immunoassay kit for the qualitative determination of Human HAV antibody in serum and plasma (EDTA, heparin, citrate).

Catalog number: ARG82303

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INTRODUCTION

Hepatitis A is an infectious disease of the liver caused by Hepatovirus A (HAV). Many cases have few or no symptoms, especially in the young. The time between infection and symptoms, in those who develop them, is between two and six weeks. When symptoms occur, they typically last eight weeks and may include nausea, vomiting, diarrhea, jaundice, fever, and abdominal pain. Around 10–15% of people experience a recurrence of symptoms during the six 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. Shellfish which have not been sufficiently cooked are a relatively common source. 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. [Wikipedia Hepatitis A]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique developed for detection and quantitation of the antibodies to HAV antibody in human serum and plasma samples. HAV antigen has been pre-coated onto a microtiter plate. Controls or samples and HRP-conjugated HAV

antibody are pipetted into the wells. HAV antibodies in controls or samples are competing the bind site of HAV antigen on the plate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and the color developed is inversely proportional to the amount of anti-HAV bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Opened kits retain activity for 4 weeks if stored as described below. Use the kit before expiration date.

Component	Quantity	Storage information
HAV Ag-coated microplate	8 X 12 strips	4°C
Positive Control	1 ml (Ready-to-use)	4°C
Negative control	1 ml (Ready-to-use)	4°C
HRP-conjugated anti-HAV antibody	12 ml (Ready-to-use)	4°C
20X Wash Buffer	55 ml	4°C
TMB substrate solution A	12ml	4°C (Protect from light)
TMB substrate solution B	12ml	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-690 nm as reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water.
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- This reagent kit is for professional use only.
- Store the kit at 4°C at all times. Microtiter wells must be stored at 2 - 8°C. Once the foil bag has been opened, care should be taken to close it tightly again and used it within 60 days after opening.
- Return all reagents to 4°C immediately after use. Opened kits retain activity for 4 weeks if stored as described above.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all kit reagents and samples to room temperature (20-28°C) before assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use.

Do not induce foaming.

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- The controls, conjugate solution and specimens should be regarded as potential health hazards. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures may include the wearing of protective gloves and avoiding the generation of aerosols.
- Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the local procedures for potential bio-hazard control.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.
b) Samples containing sodium azide should not be used in the assay.
c) Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.
d) Frozen specimens must be thoroughly thawed and mixed homogenously before testing.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled or deionized water (Do not use tap water) to yield 1X Wash buffer, mix well. (E.g. 50 ml of 20X Wash buffer + 950 ml of distilled water) The diluted Wash buffer is stable for 1 weeks at 2°C to 8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (20- 28°C) before use, each vial should be mixed thoroughly without foaming prior to use. Samples and controls should be assayed in duplicates. Each run of assay needs its own negative controls, positive controls and blank wells.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 10 µl of positive control, negative control and samples in duplicate into appropriate wells. Keep two wells empty as blank.
3. Add 100 µl of HRP-conjugated anti-HAV antibody into each well (except blank well, do not touch the well wall to prevent contamination). Gently tap the plate to mix well. Cover wells and incubate for 60 minutes at 37°C.
4. Aspirate each well and wash, repeating the process 5 times for a total 6 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 10 sec before remove. 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.

5. Prior to use, **mix equal volumes of TMB substrate solution A and TMB substrate solution B** in a clean container. Add **100 µl** of the **TMB mixture solution** to each well including the blank well.
6. Cover the plate and incubate for **20-30 minutes** at **37°C in dark**. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated standard solutions; the other wells show no obvious color).
7. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
8. Read the OD with a microplate reader at **450nm** immediately. It is recommended read the absorbance within **15 minutes** after adding the stop solution.
9. The color of the blank should be colorless to light yellowish; otherwise, the test results are invalid. Substrate blank absorbance value must be less than 0.100.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of controls and samples.

2. The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≤ 0.1
- The MV of the measured OD value for the Negative Control (NC) must be ≥ 0.4
- The NC-PC value must be ≥ 0.3

3. Calculation of the Cutoff Value:

Cut off Value = (NC + PC)/2

4. Retest zone

Retest zone= Cut off Value ± 10%

For example: If the Cut off Value is 0.6.

Then the Retest zone = [0.6 – (0.6*0.1)] to [0.6 + (0.6*0.1)]

= 0.54 to 0.66

INTERPRETATION OF TEST RESULTS

Qualitative: (Positive – Negative)

The results are valid if the following criteria are met:

A sample with the OD > Cut off Value is negative

(Specific antibodies to HAV could not be detected)

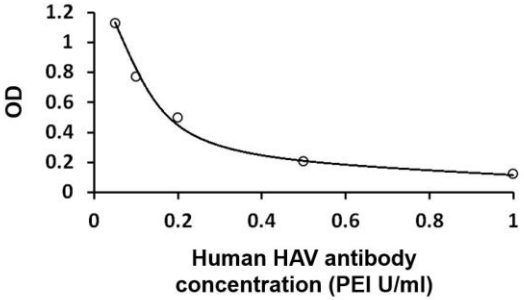
A sample with the OD ≤ Cut off Value is positive

(Specific antibodies to HAV were detected)

If the data is within the Retest zone, the test is suggested to 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.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please the data is for demonstration only and this kit does not need standard.



QUALITY ASSURANCE

Analytical sensitivity

0.121 PEI U/ml ≒ 0.157 IU/ml.

Precision

The CV values of intra-assay was < 10% and inter-assay was < 20%.

Traceability

Concentration of Anti-HAV Positive Control = 7 ±4 PEI U/ml = 9.1 ±5.2 IU/ml.