Hepatitis A virus Ab ELISA Kit

Catalog Number KA0284
96 assays
Version: 08

Intended for research use only
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Introduction

Intended Use

Hepatitis A virus Ab ELISA Kit is an enzyme immunoassay kit for qualitative detection of Antibody to Hepatitis A (Anti-HAV) in human serum or plasma.

Background

The hepatitis A virus (HAV) is a single-stranded RNA-containing virus without an envelope and with a diameter of 27 nm that belongs to the family of Picornaviridae. Hepatitis A - the most common form of acute viral hepatitis - is an infection of fecal-oral transmission produced in humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice.

Total anti-HAV and especially IgM anti-HAV is positive at the onset of a hepatitis A infection. After natural infection, anti-HAV-IgG antibodies can usually be detected for a lifetime providing protection against the disease. The detection of anti-HAV is indicative of current immunity and helps in deciding whether active immunization should be supplied by vaccination or immunoglobulins should be administered for post-exposure prophylaxis in at-risk situations.

Hepatitis A virus Ab ELISA Kit is a fast test for the qualitative detection of antibodies to Hepatitis A virus in serum or plasma specimens. This is an enzyme linked immunosorbent assay (ELISA) which utilizes HAV Ag on microtiter wells and human peroxidase-conjugated Anti-HAV in a competition principle to detect Anti-HAV levels in serum or plasma.

Principle of the Assay

Hepatitis A virus Ab ELISA Kit is a solid-phase enzyme immunoassay (ELISA=enzyme-linked immunosorbent assay) based on a competitive principle. The solid phase of the microtiter plate is made of polystyrene wells coated with HAV Ag and the liquid phase of human peroxidase conjugated Anti-HAV.

When a serum or plasma specimen containing Anti-HAV is added to the HAV Ag-coated wells together with the human peroxidase conjugated Anti-HAV and incubated, a competition will take place for the binding to the HAV Ag on the wells. (HAV Ag) - (Anti-HAV • Peroxidase) complex and/or (HAV Ag)-(Anti-HAV) complex will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. Due to the competitive principle a color develops inversely proportional to the amount of Anti-HAV bound to HAV Ag deriving from the specimen. The Peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAV Ag Plate</td>
<td>Microtiter Plate Coated with HAV Antigen.</td>
<td>96 wells</td>
</tr>
<tr>
<td>Anti-HAV-Peroxidase Solution</td>
<td>Anti-HAV (mouse monoclonal) -Peroxidase (horseradish) conjugate dissolved in buffer with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.</td>
<td>12 mL</td>
</tr>
<tr>
<td>Anti-HAV Positive Control</td>
<td>Human plasma positive for antibody to HAV in buffer with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.</td>
<td>1 mL</td>
</tr>
<tr>
<td>HA Negative Control</td>
<td>Human plasma non-reactive for antibody to HAV with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.</td>
<td>1 mL</td>
</tr>
<tr>
<td>TMB Substrate Solution A</td>
<td>3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate Solution B</td>
<td>Citric Acid Buffer containing H₂O₂.</td>
<td>12 mL</td>
</tr>
<tr>
<td>Conc. Washing Solution D (20X)</td>
<td>Concentrated phosphate buffer with Tween-20.</td>
<td>58 mL</td>
</tr>
<tr>
<td>2N H₂SO₄</td>
<td>2N H₂SO₄ (Sulfuric Acid)</td>
<td>12 mL</td>
</tr>
</tbody>
</table>

Accessories: (provided as needed)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive slips</td>
<td>1</td>
</tr>
<tr>
<td>Black cover</td>
<td>1</td>
</tr>
</tbody>
</table>

Storage Instruction

- The kit must be stored at 2 to 8°C. Do not freeze.
- Strips of the plate should be used within 1 month after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.
- Return the reagents to 2 to 8°C immediately after use.
- Washing Solution D (20x) Concentrate should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a 37°C water bath till the crystal is dissolved.
Materials Required but Not Supplied

☑️ 10 µL, 100 µL micropipettes and tips are needed
☑️ Incubator or water bath with temperature control at 37±1°C
☑️ Plate washing equipment.
☑️ ELISA microwell reader: Dual wavelength 450 nm with 620-690 nm as reference wavelength, bandwidth 10 nm

Precautions for Use

- Procedural Guidelines
  ☑️ For professional use only.
  ☑️ Bring all kit reagents and samples to room temperature (20 to 30°C) and mix gently before use.
  ☑️ Do not use reagent beyond its expiration date.
  ☑️ Do not interchange reagents between different lots.
  ☑️ Do not pipette in the mouth.
  ☑️ Do not smoke or eat in areas where specimens or reagents are handled.
  ☑️ The positive control, negative control, conjugate solution and specimens should be regarded as potential hazards to health. They shall be used and discarded according to the user’s laboratory safety procedures. Such safety procedures probably shall include wearing protective gloves and avoiding aerosols generation.
  ☑️ Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory's practice for potential bio-hazard control.
  ☑️ Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:
    Both liquid and solid waste should be autoclaved maintaining 121°C for at least 30 minutes.
    Solid waste can also be incinerated.
    Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%
    Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.
  ☑️ 2N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.
  ☑️ TMB substrate solution A contains an organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.
Assay Protocol

Reagent Preparation

- Washing solution
  Dilute Washing Solution D (20x) Concentrate with distilled or de-ionized water to 1:20 dilution. Do not use tap water.
- Automatic or Semi-automatic Plate Washer
  Any commercial automatic microplate washer or other liquid aspirating/dispensing devices can be used for washing purpose. The user should test the devices to determine the proper volume of water and wash cycles to insure proper washing. We suggested 6 wash cycles with at least 350 μL per well per wash and soaking for 10 seconds is necessary.
- Manual Plate Wash
  Cover the reaction plate with an absorbent paper. Invert the plate and allow the liquid absorb onto the absorbent paper, then return the plate back to upright position. Fill each well with 350 μL of washing buffer. Aspirate the water after soaking 10 seconds. Repeat this procedure 6 times. Blot dry by inverting the plate and tapping firmly onto absorbent paper. All residual washing buffer should be blotted dry.
  **WARNING:** Improper washing can cause false

Sample Preparation

- Specimen Collection and Preparation for Analysis
  - Either serum or plasma can be used with this kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.
  - The specimen must not contain any compounds of AZIDE, which inhibits the peroxidase activity.
  - Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.
  - No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques.

- Stability and storage
  - Specimens must be stored at 2 to 8°C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20°C. Storage in self-defrosting freezer is not recommended.
  - Frozen specimens must be thoroughly thawed and mixed homogenously before test.
  - Avoid multiple freeze-thaw procedures
**Assay Procedure**

Assay process can be performed by an automatic EIA micro-plate immunoanalyzer. Please set up the program according to the following test procedure.

1. Bring all reagents and specimens to room temperature (20 to 30°C) before assay. Adjust water bath or incubator to 37±1°C.
2. Prepare the needed number of wells, including 2 wells for blanks, 3 wells for Negative Control, 2 wells for Positive Control, and one well for each specimen.
   Reserve 2 wells for blanks (Do not add any specimen or conjugate).
   Add 10 µL of each control or specimen to the appropriate wells of HAV Ag coated plate, except the 2 blanks.
3. Add 100 µL of Anti-HAV-Peroxidase solution to each of the above wells except the 2 blanks. Gently tap the plate.
4. Seal the plate with an adhesive slip.
5. Incubate the reaction plate in 37±1°C water bath or incubator for one hour.
6. At the end of the incubation period, remove and discard the adhesive slip and wash the plate.
7. Choice one of the following two methods for color development:
   - Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use.
     Add 100 µL of the mixture solution to each well including the 2 blank wells.
   - Add 50 µL of TMB Substrate Solution A first, then add 50 µL of TMB Substrate Solution B into each well including the 2 blanks. Mix well gently.
8. Cover the plate with black cover and incubate at room temperature (20-30°C) for 30 minutes.
9. Stop the reaction by adding 100 µL of 2N H₂SO₄ to each well including the blank.
10. Determine the absorbance of controls and test specimens within 15 minutes with a photometer at 450 nm with a selected reference wavelength within 650 nm. Use the blank well to blank the photometer.

**Note:**

- *Use a new pipette tip for each sampling to avoid cross-contamination*
- *Each plate needs its own negative controls, positive controls and blank wells.*
- *Do not touch the cuvette wall for preventing contamination.*
- *TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mixing. The mixture should be avoided from intense light.*
- *The color of the blank should be colorless to light yellowish; otherwise, the test result is invalid. In this case the test must be repeated.*
- *Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.*
Data Analysis

Calculation of Results

- Calculation of the NCx (Mean Absorbance of Negative Control)

  Example:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.263</td>
</tr>
<tr>
<td>2</td>
<td>1.305</td>
</tr>
<tr>
<td>3</td>
<td>1.290</td>
</tr>
</tbody>
</table>

  $NCx = \frac{(1.263 + 1.305 + 1.290)}{3} = 1.286$

  NCx must be $\geq 0.40$, otherwise, the test is invalid.

- Calculation of PCx (Mean Absorbance of Positive Control)

  Example:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.054</td>
</tr>
<tr>
<td>2</td>
<td>0.060</td>
</tr>
</tbody>
</table>

  $PCx = \frac{(0.054 + 0.060)}{2} = 0.057$

  PCx must be $\leq 0.10$, otherwise, the test is invalid.

- Calculation of the N-P Value

  $N-P = NCx - PCx$

  Example:

  $N - P = 1.286 - 0.057 = 1.229$

  N-P Value must be $\geq 0.30$, otherwise, the test is invalid.

- Calculation of the Cutoff Value

  Cutoff Value = $(NCx + PCx)/2$

  Example:

  Cutoff Value = $(1.286 + 0.057)/2 = 0.672$

- Calculation of the Retest Range

  Retest Range = Cutoff Value ± 10%

  Example: Cutoff Value = 0.672

  Retest Range = $(0.672 - 0.067)$ to $(0.672 + 0.067) = 0.605 \sim 0.739$
Note:

- Specimens with O.D. values GREATER than the Cutoff Value are considered non-reactive for Anti-HAV.
- Specimens with O.D. values LOWER than or EQUAL to the Cutoff Value are considered reactive for Anti-HAV.
- If the data is within the 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.

- Flow chart of the test procedure

Incubation: 37 °C, 1 hr

Add 10 µL controls (3x NC, 2x PC) and add 10 µL Specimen Diluent into wells.
Reserve 2 wells for blank.

↓

Add 100 µL of the Anti-HAV Peroxidase Solution to each well containing Specimen Diluent. (blank test wells do not add)

↓

Incubate in +37 ±1 °C for 1 hour

(Choose one of the following two methods for color development)

Mix equal volumes of TMB Substrate Solution A and B. Add 100 µL of the mixed solution to wells.

Add 50 µL of TMB Substrate Solution A to wells and then add 50 µL of TMB Substrate Solution B. Mix well, gently.

Incubate at R.T. for 30 minutes.

↓

Add 100 µL of 2N H₂SO₄ into each well.

↓

Determine absorbance at 450 nm or 450/650 nm
Resources

References


