Hepatitis B virus core antigen Ab ELISA Kit

Catalog Number KA0288
96 assays
Version: 03

Intended for research use only
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Introduction

Intended Use

Hepatitis B virus core antigen Ab ELISA kit is an enzyme immunoassay kit for qualitative detection of Hepatitis B virus core antigen (HBcAg) in human serum or plasma.

Background

The hepatitis B virus (HBV) consists of an external envelope (HBsAg) and an inner core (HBcAg). In acute HBV infection, antibody to HBcAg (Anti-HBc total) is detectable in serum or plasma shortly before clinical symptoms and slightly after the appearance of HBsAg. In cases in which HBV infection resolves, anti-HBc appears in blood during the window period following loss of HBsAg and prior to the development of antibody to HBsAg (anti-HBs). Anti-HBc is found in acute and chronic hepatitis B patients and also indicates past resolved infection. Therefore, detection of anti-HBc is indicative of exposure to HBV and thus of infection by this virus. Further testing of HBV serological markers is required to define the specific disease state.*1-6

Hepatitis B virus core antigen Ab ELISA kit is a fast test for the qualitative detection of the presence of antibodies to HBcAg in serum or plasma (heparin, citrate or EDTA) specimens. The test utilizes HBcAg on microtiter wells and human peroxidase-conjugated Anti-HBc in a competition principle to detect Anti-HBc levels in serum or plasma.

Specimens with absorbance values greater than 1.1 x Cutoff Value are considered NEGATIVE for Anti-HBc. Specimens with absorbance values less than 0.9 x Cutoff Value are considered POSITIVE for Anti-HBc.

The test has to be repeated in duplicate for specimens with absorbance value within the retest range (Cutoff Value ± 10 %) and interpreted as above.

If the absorbance of any of the specimens retested in duplicate is still within the retest range, it is suggested to test follow-up samples of the patient.

Principle of the Assay

Hepatitis B virus core antigen Ab ELISA kit is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immune assay) - based on a competitive principle. The solid phase of the microtiter plate is made of polystyrene wells coated with HBcAg and the liquid phase of human peroxidase conjugated Anti-HBc.

When a serum or plasma specimen containing Anti-HBc is added to the HBcAg-coated wells together with the human peroxidase conjugated Anti-HBc and incubated, a competition will take place for the binding to the HBcAg on the wells. (HBcAg)-(Anti-HBc • Peroxidase) complex and/or (HBcAg)-(Anti-HBc) 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-HBc bound to HBcAg 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*8.

The above test principle is shown also in the following figure.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBcAg Plate</td>
<td>Microtiter plate coated with HBcAg.</td>
<td>1 plate</td>
</tr>
<tr>
<td>Anti-HBc - Peroxidase Solution</td>
<td>Anti-HBc (human) - Peroxidase conjugate dissolved in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>8 mL</td>
</tr>
<tr>
<td>Anti-HBc Positive Control</td>
<td>Anti-HBc positive serum in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>HB Negative Control</td>
<td>Serum non-reactive for HBV markers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>2 mL</td>
</tr>
<tr>
<td>TMB Substrate Solution A</td>
<td>0.6 mg/mL of 3,3’,5,5’-tetramethylbenzidine (TMB) in an organic base.</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate Solution B</td>
<td>Citrate acid buffer containing 0.03% 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>X1</td>
</tr>
<tr>
<td>Black cover</td>
<td>X1</td>
</tr>
</tbody>
</table>

Storage Instruction

- The kit must be stored at 2-8°C. Do not freeze.
- Strips of the plate should be used within one month after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and tapped the opening tightly.
- Return the reagents to 2-8°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 crystal dissolved.
Materials Required but Not Supplied

- 50 µL, 100 µL micropipettes and tips are needed
- Incubator or water bath with temperature control at 37°C
- Plate washing equipment.
- ELISA microwell reader
- Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

Precautions for Use

- **Procedural Guidelines**
  - This kit is for medical technicians or physicians used only.
  - This reagent kit is for in vitro diagnosis only.
  - Bring all kit reagents and samples to room temperature (20-30°C) and mix gently before use.
  - Do not use kit beyond its expiration date.
  - Do not interchange reagents between different lots.
  - Reagents must be protected from microbial contamination.
  - The positive and negative control sera have been inactivated, however, for safety reason, they must be treated as infectious material.
  - Do not smoke or eat in areas where specimens or reagents are handled.
  - Do not pipette by mouth.
  - Wear gloves when handling reagents or specimens, and wash hands thoroughly afterwards.
  - Infectious specimens and nonacid containing spills should be wiped up thoroughly with 5% sodium hypochlorite.
  - All waste materials should be properly disinfected before disposal. Both liquid and solid waste should be autoclaved for at least 1 hour at 121°C. Solid waste can also be incinerated. Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%. Liquid waste containing acid must be neutralized before similar treatment and should stand for 30 minutes to obtain effective disinfection.
  - TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes. Avoid contact of TMB substrate solution and sulfuric acid with 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.

- Plate Washing Procedure

  ✓ 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 results.

Sample Preparation

- Specimen Collection and Preparation for Analysis

  ✓ Either serum or plasma can be used with this diagnostic kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Also, clots must be removed.

  ✓ Specimens must be stored at 2-8°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.

  ✓ Avoid multiple freeze-thaw procedures.

  ✓ Frozen specimens must be thawed thoroughly and mixed before test.

  ✓ Specimens must not contain any sodium azide, which inhibits the peroxidase activity.
Assay Procedure

1. Bring all reagents and specimens to room temperature (20-30°C) before assay. Adjust a water bath or incubator to 37±1°C.

2. Reserve 2 wells for blanks. Add 50 μL of each control or specimen to appropriate wells of reaction plate (3 Negative Controls and 2 Positive Controls).

3. Add 50 μL of Anti-HBc • Peroxidase Solution to each well except 2 blanks.
   
   **NOTE:** Use a new pipette tip for each sampling to avoid cross contamination.

4. Gently tap the plate.

5. Remove the protective backing from the Adhesive Slip and press it on the reaction plate, so that it is tightly sealed.

6. Incubate the reaction plate in a 37±1°C water bath for 1 hour.

7. At the end of the incubation period, remove and discard the Adhesive Slip and wash plate by following "PLATE WASHING PROCEDURES".

8. Choice one of the following two methods for color development:

   **NOTE:** TMB Substrate Solution A should be colorless to light blue, otherwise, should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mix. The mixture should be avoided from intense light.

   ✓ Mix equal volume 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 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 2 blanks. Mix well gently.

9. Cover the plate with Black Cover and incubate at room temperature for 30 minutes.

10. Stop the reaction by adding 100 μL 2N H₂SO₄ to each well including 2 blanks.

11. Determine absorbance of Controls and test specimens within 15 minutes with a precision spectrophotometer at 450 nm or 450/650 nm (450 nm reading wavelength with 650 nm reference wavelength). Use the lighter color of two blank wells to blank the spectrophotometer.

   **NOTE:** The color of the blank should be colorless to pale yellow, otherwise, the test must be repeated.
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>0.939</td>
</tr>
<tr>
<td>2</td>
<td>0.944</td>
</tr>
<tr>
<td>3</td>
<td>0.925</td>
</tr>
</tbody>
</table>

NCx = (0.939 + 0.944 + 0.925) / 3 = 0.936
NCx must be ≥ 0.4, otherwise, the test run is invalid.

Calculation of the 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>0.068</td>
</tr>
<tr>
<td>2</td>
<td>0.052</td>
</tr>
</tbody>
</table>

PCx = (0.068 + 0.052) / 2 = 0.060
PCx must be ≤ 0.1, otherwise, the test run is invalid.

Calculation of the N - P Value
N - P = NCx – PCx
Example:

N – P = 0.936 – 0.060 = 0.876
N - P Value must be ≥ 0.3, otherwise, the test run is invalid.

Calculation of the Cutoff Value
Cutoff Value = 0.4 NCx + 0.6 PCx
Example:

Cutoff Value = (0.4 x 0.936) + (0.6 x 0.060) = 0.410

Calculation of the Retest Range
Retest Range = Cutoff Value ± 10%
Example: Cutoff Value = 0.410
Retest Range = (0.410 - 0.041) to (0.410 + 0.041) = 0.369 to 0.451
Note:

✓ Specimens with absorbance values greater than the cutoff value are considered negative for Anti-HBc.

✓ Specimens with absorbance value less than or equal to the cutoff value are considered positive for Anti-HBc.

✓ If the signal/cut-off ratio is within retest range, the test must be repeated in duplicate and interpreted as above.
Resources

Plate Layout

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
</table>

This table represents a plate layout for resources, with rows and columns labeled from 1 to 12.