Hepatitis B surface antigen Ab ELISA Kit

Catalog Number KA0287
96 assays
Version: 18

Intended for research use only
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Introduction

Intended Use

Hepatitis B surface antigen antibody ELISA kit is an enzyme immunoassay kit for qualitative detection of antibody to Hepatitis B surface antigen (Anti-HBs) in human serum or plasma.

Background

Hepatitis B (HB) is a disease caused by viral infection. The route of infection can be an improper needle puncture or blood transfusion.

Hepatitis B has become a significant problem for public health management. Almost one in every ten adults, who has been infected by Hepatitis B Virus (HBV), develops some forms of chronic liver disease and become a long-term carrier of HBV.

Hepatitis B is an immune disease. Invasion of the human body by HBV induces autoimmune responses, which damage the liver. The components of the virus (antigen) and the host responses (antibody) have often been used as diagnostic tools. There are six types of immunological markers of HBV: Hepatitis B surface antigen (HBsAg), Hepatitis B core antigen (HBcAg), Hepatitis B e antigen (HBeAg) and their respective antibodies. The antibody to HBsAg or anti-HBs however is the last marker to show up in the serum. The presence of anti-HBs generally means the conclusion of and immune to HBV infection.

Principle of the Assay

Hepatitis B surface antigen antibody ELISA kit adopts the sandwich principle as the basis of the assay. The wells of polystyrene microplate have been coated with HBsAg. When the test sample containing anti-HBs is incubated with HBsAg • Peroxidase solution in the wells, HBsAg – Anti-HBs – HBsAg • Peroxidase complexes are formed on the wells. After washing to remove unbound materials, a peroxidase substrate is added and color develops in proportion to the amount of Anti-HBs bound. The level color is greatest in the presence of Anti-HBs and falls from its level with decreasing concentrations of Anti-HBs in the sample.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg Plate</td>
<td>96-well Microtiter plate coated with HBsAg.</td>
<td>1 plate</td>
</tr>
<tr>
<td>HBsAg • Peroxidase Solution</td>
<td>HBsAg (Human) • HRPO conjugate, diluted in buffer with protein stabilizers.</td>
<td>8 mL</td>
</tr>
<tr>
<td></td>
<td>Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td></td>
</tr>
<tr>
<td>Anti-HBs Positive Control</td>
<td>Anti-HBs serum diluted in buffer with protein stabilizers.</td>
<td>1.5 mL</td>
</tr>
<tr>
<td></td>
<td>Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td></td>
</tr>
<tr>
<td>HB Negative Control</td>
<td>Serum non-reactive for HBV markers, but contains protein stabilizers.</td>
<td>2 mL</td>
</tr>
<tr>
<td></td>
<td>Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td></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>Citrate Acid Buffer containing H₂O₂</td>
<td>12 mL</td>
</tr>
<tr>
<td>Conc. Washing Solution D (20x)</td>
<td>Phosphate buffer with Tween-20</td>
<td>58 mL</td>
</tr>
<tr>
<td>2 N Sulfuric acid</td>
<td>2 N 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 2-8°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.
- Automatic microplate washing equipment.
- Precision ELISA Reader: capable for 450 nm or 450 nm/650 nm wavelength.
- Special order of anti-HBs standards is needed for Quantitative Assay.

Precautions for Use

- This reagent kit is for research use 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

✓ Plate Washing Procedure
   Note: Dilute Washing Solution D (20X) Concentrate with distilled or deionized 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 0.35 mL 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 and then return the plate back to upright position. Fill each well with 0.35 mL 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.
   Note: Improper washing can cause false.

Sample Preparation

✓ Specimen Collection and Storage
- 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. 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.
- Pretreatment of sample is needed if the sample containing substances that may interfere with the assay.
- 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 to 30°C) before assay.
2. Reserve 2 wells for blank. Add 50 µL of each control or specimen to appropriate wells of reaction plate (3 Negative Controls and 2 Positive Controls).
   *NOTE: Use a new pipette tip for each sampling to avoid cross-contamination.*
3. Add 50 µL of HBsAg • Peroxidase solution to each well except the blank.
4. Gently tap the plate.
5. Remove the protective backing from the Adhesive Slip and press it onto the reaction plate, so that it is tightly sealed.
6. Incubate the reaction plate in a 37±1°C 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 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 blank well.
   - Add 50 µL of TMB Substrate Solution A first, and then add 50 µL of TMB Substrate Solution B into each well including 2 blanks. Mix well gently.
9. Cover the plate with a black cover and incubate at room temperature (20-30°C) for 30 minutes.
10. Stop the reaction by adding 100 µL of 2 N H₂SO₄ to each well including 2 blanks.
11. Determine the absorbance of Controls and test specimens within 15 minutes with a precision photometer 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:*
   1. *The color of the blank should be colorless to pale yellow; otherwise, the test must be repeated.*
   2. *The absorbance of Blank wells should be LESS than 0.100.*
Data Analysis

Calculation of Results

✓ Calculation of the NCx (Negative Control Mean Absorbance)
  Example:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
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<tbody>
<tr>
<td>1</td>
<td>0.024</td>
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<tr>
<td>2</td>
<td>0.023</td>
</tr>
<tr>
<td>3</td>
<td>0.025</td>
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</tbody>
</table>

NCx = (0.024 + 0.023 + 0.025) / 3 = 0.024
NCx must be ≤ 0.2, otherwise, the test is invalid.

✓ Calculation of the PCx (Positive Control Mean Absorbance)
  Example:

<table>
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<tr>
<th>Sample No.</th>
<th>Absorbance</th>
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<tbody>
<tr>
<td>1</td>
<td>1.432</td>
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<tr>
<td>2</td>
<td>1.508</td>
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</tbody>
</table>

PCx = (1.432 + 1.508) / 2 = 1.470
PCx must be ≥ 0.5, otherwise, the test is invalid.

✓ Calculation of the P - N Value
  \[ P - N = PCx - NCx \]
  Example: \[ P - N = 1.470 - 0.024 = 1.446 \]
  P - N Value must be ≥ 0.3, otherwise, the test is invalid.

✓ Calculation of the Cutoff Value
  Cutoff Value = NCx + 0.025
  Example: Cutoff Value = 0.024 + 0.025 = 0.049

✓ Calculation of the Retest Range
  Retest Range = Cutoff Value ± 10%
  Example: Cutoff Value = 0.049 ± 10%
  Retest Range = (0.049 - 0.005) to (0.049 + 0.005) = 0.044 to 0.054
Interpretation of Results

- Specimens with absorbance values LESS than the Cutoff Value are considered NON-REACTIVE for Anti-HBs by the criteria of Hepatitis B surface antigen antibody ELISA.
- Specimens with absorbance values GREATER than the Cutoff Value is considered REACTIVE for Anti-HBs.
- If the data is within the Retest Range, the test must be repeated in duplicate and interpreted as above.

Flow chart of the test procedure

1. Add 50 µL of Controls (3 NC, 2 PC) and 50 µL per Specimen into wells. Reserve 2 wells for blank.
2. Add 50 µL of HBsAg • Peroxidase solution to each well except the blank.
3. Incubate the plate at 37 ± 1°C for 60 minutes.
4. Wash the plate.
5. Mix the TMB Substrate Solution A and B by the equal volume. Add 100 µL of the mixed substrate solution to wells.
6. Add 50 µL of TMB Substrate Solution A to wells and then add 50 µL of TMB Substrate Solution B. Carefully mix well.
7. Incubate at RT for 30 minutes.
8. Add 100 µL of 2 N H₂SO₄ into each well.
9. Determine absorbance within 15 minutes using 450 nm or 450/650 nm.
Resources

References


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