Hepatitis B surface antigen ELISA Kit

Catalog Number KA0286
96 assays
Version: 12

Intended for research use only
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Introduction

Intended Use

Hepatitis B surface antigen ELISA kit is a sandwich enzyme immunoassay kit for qualitative detection of Hepatitis B surface antigen (HBsAg) in human serum or plasma.

Background

The hepatitis B surface antigen (HBsAg) is the first marker that appears in the blood following infection with hepatitis B virus (HBV) some days or weeks before clinical symptoms manifest. It is a lipoprotein polypeptide which constitutes the external envelope of the HB virus. The detection of HBsAg in human serum or plasma indicates an ongoing HBV infection, either acute or chronic. Testing of additional HBV markers is needed to define the specific disease state. HBsAg assays are used not only to diagnose HBV infections but also to monitor the course of the disease and the efficacy of antiviral therapy.

Hepatitis B surface antigen ELISA kit is a fast test for the qualitative detection of the presence of HBsAg in serum or plasma (heparin, citrate or EDTA) specimen. The test utilizes monoclonal and polyclonal (anti-guinea pig) antibodies to selectively detect elevated levels of HBsAg in serum or plasma.

Specimens which are non-reactive by Hepatitis B surface antigen ELISA kit are considered negative for HBsAg.

Specimens with positive reaction should be retested in duplicate.

In case of a reactive repeat reaction, the specimen should be confirmed for HBsAg reactivity with validated confirmatory reagents. Only confirmed positive specimens are considered to contain HBsAg.

Principle of the Assay

Hepatitis B surface antigen ELISA Kit is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immuno-sorbent assay) based on the “sandwich principle”.

The solid phase of the microtiter plate is made of polystyrene wells coated with mouse monoclonal antibodies specific for HBsAg; whereas guinea pig polyclonal antibody purified by affinity chromatography is used to prepare the anti-HBs+ peroxidase (horseradish) conjugate in the liquid-phase.

When a serum or plasma specimen containing HBsAg is added to the anti-HBs antibody-coated wells together with the peroxidase conjugated anti-HBs antibody and incubated, an antibody-HBsAg-antibody-peroxidase 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. A color develops in proportion to the amount of HBsAg boud to Anti-HBs. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.
A. Specimen containing HBsAg:
   1. Plate well (Anti-HBs) + specimen (HBsAg) + Anti-HBs•HRPO → Anti-HBs•HBsAg•(Anti-HBs•HRPO) sandwich complex
   2. Sandwich complex + TMB substrate solution (colorless) → Light blue to blue color
   3. Add sulfuric acid to stop the color development → Read OD at 450 nm (reference wavelength 650 nm)

B. Specimen without HBsAg:
   1. Plate well (Anti-HBs) + specimen (no HBsAg) + Anti-HBs•HRPO → Anti-HBs (on the well)
   2. Anti-HBs (on the well) + TMB substrate solution (colorless) → Colorless to light blue color
   3. Add sulfuric acid to stop the color development → Read OD at 450 nm (reference wavelength 650 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>Anti-HBs Plate</td>
<td>Microtiter plate coated with mouse monoclonal anti-HBs.</td>
<td>1 plate</td>
</tr>
<tr>
<td>Anti-HBs • Peroxidase</td>
<td>Polyclonal Anti-HBs • HRPO conjugate, diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal. Dye: phenol red.</td>
<td>8 mL</td>
</tr>
<tr>
<td>HBsAg Positive Control</td>
<td>Inactivated human serum positive for HBsAg (8 ng/mL) but non-reactive for anti-HCV and anti-HIV1/2, diluted 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, anti-HCV and anti-HIV1/2, diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>2 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>Citrate Acid Buffer containing H₂O₂.</td>
<td>12 mL</td>
</tr>
<tr>
<td>Conc. Washing Solution D</td>
<td>Concentrated Phosphate buffer with Tween-20</td>
<td>58 mL</td>
</tr>
<tr>
<td>2N H₂SO₄</td>
<td>2N 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 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**

- 50 µL, 100 µL micropipettes and tips are needed.
- Water bath or incubator 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**

- This reagent kit is for professional use only.
- Bring all kit reagents and samples to room temperature and mix carefully before use.
- Do not use reagent beyond its expiration date.
- Do not interchange reagents between different lots.
- Reagents must be protected from microbial contamination.
- Do not put pipette in mouth.
- Do not smoke or eat in areas where specimens or reagents are handled.
- All kit components and specimens should be regarded as potential health hazards. It should be used and discarded according to your laboratory’s safety procedures. Such safety procedures probably include the wearing of protective gloves and avoiding the use of aerosols.
- Although all human sourced material are tested non reactive for Anti-HCV and Anti-HIV, and inactivated at 56°C for one hour, the reagent shall be handled as potential infectious material.
- Potential infectious specimens and non-acid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with your practice for potential bio-hazard control.
- Prior to disposing used specimens and kit reagents as general waste; it should be treated in accordance with the local practice of potential bio-hazardous waste or treated as follows:
  - Both liquid and solid waste should be autoclaved at 121°C for at least 1 hour.
  - 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, flush immediately with abundant amounts of water. In case of inhalation, find fresh air and seek medical attention in case of pain.
- TMB substrate solution A contains 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 obtain a 1:20 dilution. Do not use tap water.

- Plate Washing Procedure.
  1. Plate washer with overflow aspirating function: 6 cycles with at least 0.5 mL washing buffer per well per cycle and soaking for 20 seconds per cycle.
  2. Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.

✓ WARNING: Improper washing can cause false

Sample Preparation

✓ Specimen Collection and Preparation for Analysis
- Either serum or plasma specimens can be used with this test kit. Whole blood specimen 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 sera and microbial-contaminated specimens should not be used.

✓ Stability and storage:
- Specimens must be stored at 2 to 8°C and avoid 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

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. Reserve two wells for blank. Add 50 µL of each control or specimen to appropriate wells of the microtiter plate (3 Negative Controls and 2 Positive Controls).
3. Add 50 µL of Anti-HBs•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 37±1°C water bath or incubator for 80 minutes.
7. At the end of the incubation period, remove and discard the Adhesive Slip and wash the plate by “Plate Washing Procedure”.
8. Select 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 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 the blank. Mix well carefully.
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 2N H₂SO₄ to each well including the blank.
11. Determine the absorbance of Controls and test specimens within 30 minutes with a precision spectrophotometer at 450/650 nm (450 nm reading wavelength with 650 nm reference wavelength). Use the blank well to blank spectrophotometer.

Note:
✓ Use a clean pipette tip for each sampling to avoid cross-contamination.
✓ Each plate needs respective negative controls, positive controls and blank well.
✓ Do not touch the wall of the plate wells to prevent 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 mix. The mixture should be kept away from intense light.
✓ The color of the blank should be colorless to light yellow; otherwise, the test results are invalid.
✓ Substrate blank: absorbance value must be less than 0.100.
Data Analysis

Calculation of Results

- **Calculation of the NC**<sub>x</sub> (Mean Absorbance of Negative Control)
  
<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.052</td>
</tr>
<tr>
<td>2</td>
<td>0.023</td>
</tr>
<tr>
<td>3</td>
<td>0.025</td>
</tr>
</tbody>
</table>

  The optical density 0.052 is twice higher than 0.025, which is abnormal. In this case we ignore the number 0.052.

  \[ NCx = (0.023 + 0.025) / 2 = 0.024 \]

  NCx should be ≤ 0.1, otherwise, the test is invalid.

- **Calculation of the PC**<sub>x</sub> (Mean Absorbance of Positive Control)
  
<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.432</td>
</tr>
<tr>
<td>2</td>
<td>1.508</td>
</tr>
</tbody>
</table>

  \[ PCx = (1.432 + 1.508) / 2 = 1.470 \]

  PC should be ≥ 0.6, otherwise, the test is invalid.

- **Calculation of the P - N Value**
  
  \[ P - N = PCx - NCx \]

  Example: NCx = 0.024

  \[ PCx = 1.470 \]

  \[ P - N = 1.470 - 0.024 = 1.446 \]

  P - N Value must be ≥ 0.5, otherwise, the test is invalid.

- **Calculation of the Cutoff Value**
  
  Cutoff Value = NCx + 0.025

  Example: Cutoff Value = 0.024 + 0.025 = 0.049

Note:

- **Specimens with absorbance values LESS than the Cutoff Value are NON-REACTIVE and are considered NEGATIVE for HBsAg.**
- **Specimens with absorbance value GREATER than or EQUAL to the Cutoff Value are considered INITIALLY REACTIVE. The original specimens must be retested in duplicate.**
- **If both absorbance values in the retest are less than the cutoff value, the specimens are considered NEGATIVE for HBsAg.**
- **If in the retest at least one of the two absorbance values is GREATER than or EQUAL to the Cutoff Value then the specimens are considered as repeated HBsAg positive. The repeated positive specimen shall be confirmed with certain valid confirmatory reagents.**
## Resources

### Plate Layout

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<th>E</th>
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