Human Hepatitis B Virus Surface Antibody (HBsAb) ELISA Kit

Catalog No.: abx257498

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C for 6 months.

Application: For qualitative detection of HBsAb in Human Serum, Plasma, Tissue Homogenates and other biological fluids.

Introduction: Hepatitis B virus, abbreviated HBV, is a species of the genus Orthohepadnavirus, which is likewise a part of the Hepadnaviridae family of viruses. This virus causes the disease hepatitis B. In addition to causing hepatitis, infection with HBV can lead to cirrhosis and hepatocellular carcinoma. It has also been suggested that it may increase the risk of pancreatic cancer. The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins. These serotypes are based on a common determinant (a) and two mutually exclusive determinant pairs (d/y and w/r). The viral strains have also been divided into ten genotypes (A–J) and forty subgenotypes according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination.

Principle of the Assay
This kit is based on enzyme-linked immuno-sorbent assay technology. A 96-well plate is pre-coated with an antigen specific to HBsAb. The standards and test samples are added to the wells and washed with wash buffer. Biotin-labeled Antigen is used for detection. Streptavidin-HRP is added and unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP activity. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the HBsAb amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of HBsAb can be calculated.

1. One pre-coated 96 well plate
2. Positive control: 4 tubes
3. Negative control: 2 ml
4. Positive Control Diluent Buffer: 10 ml
5. Biotin Detection Reagent (Dilution 1:100): 120 µl
6. Detection Reagent diluent buffer: 10 ml
7. Streptavidin-HRP conjugate (SABC) (Dilution 1:100): 120 µl
8. SABC diluent buffer: 10 ml
9. TMB substrate: 10 ml
10. Stop solution: 10 ml
11. Wash buffer (25X): 30 ml
12. Plate Sealer: 5

Material Required But Not Provided
1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant.

» NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

» Store samples undiluted. Once ready to analyze, thaw samples and dilute 1:11 with sample diluent buffer.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Preparation of HRP-conjugate working solution: prepare no more than 1 hour before the experiment.

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the HRP-conjugate with the HRP-conjugate diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of HRP-conjugate into 99 µl of HRP-conjugate diluent buffer.

4. Preparation of Biotin Detection Reagent working solution: prepare no more than 1 hour before the experiment.

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the Biotin Detection Reagent with Detection Reagent diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin Detection Reagent into 99 µl of Detection Reagent diluent buffer.

5. Preparation of Streptavidin-HRP Conjugate (SABC) working solution: prepare no more than 30 min. before the experiment

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the SABC with SABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of SABC into 99 µl of SABC diluent buffer.

B. Assay Procedure

Equilibrate the SABC working solution to room temperature and TMB substrate at 37°C for 30 minutes prior to use. It is recommended to plot a standard curve for each test.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature for at least 30 min prior to use.

1. **Wash the plate twice before adding controls and samples.** Set positive/negative, test sample and control (zero) wells on the pre-coated plate respectively and record their positions.
2. Add 100 µl of positive control to each positive control well; 100 µl of negative control to each negative control well; and 100 µl of sample to each sample well. Leave the blank wells without any solution. Add the solution at the bottom without touching the sides of the well. Shake the plate gently to mix the contents.
3. Seal the plate with a cover and incubate at 37°C for 60 min.
4. Remove the cover and discard the plate contents by tapping the plate on absorbent filter papers or other absorbent material. Do not wash.
5. Add 100 µl of prepared Biotin Detection Reagent working solution into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for 60 minutes.
6. Seal the plate with a cover and incubate at 37°C for 60 min.
7. Wash the plate 3 times with wash buffer. Do not let the wells completely dry at any time.

Manual Washing: Discard the solution without touching the side walls and wash the plate five times. Fill each well completely with Wash buffer and incubate on an ELISA shaker for 2 min. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

Automated Washing: Discard the solution and wash the plate five times overfilling the wells with Wash buffer. After the final wash invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. It is recommended that the washer be set for a soaking time of 1-2 min.

8. Add 100 µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 minutes.
9. Remove the cover and wash the plate 5 times with Wash buffer. Allow the wash buffer to remain in the wells 1-2 min for each wash. Discard the washing buffer and blot the plate onto absorbent filter papers or other absorbent material.
10. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the positive control wells the reaction can be terminated.
11. Add 50 µl of Stop solution into each well (including the blank well). There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

C. Analysis

1. The average value of the positive control ≥ 0.6; the average value of the negative control ≤0.1. If results are outside these parameters, the test is invalid.
2. The critical value (CUT OFF) = the average value of the negative control + 0.025
3. Negative Result: if the OD value < CUT OFF, the sample is HBsAb negative.
4. Positive Result: if the OD value ≥ CUT OFF, the sample is HBsAb positive.

D. Precautions

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Wash buffer may crystallize and separate. If this happens, please heat the tube to dissolve.
4. It is recommended measuring each controls and samples in duplicate or triplicate.
5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components or components from a different kit.
10. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. TMB Substrate solution should also be protected from light. Equilibrate the TMB substrate at room temperature prior to use. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.
11. A pilot experiment experiment with the controls and a small number of samples is recommended to determine if sample dilution is required.
11. Keep the plate dry after opening and before use.