Human HBeAg(hepatitis B virus E Antigen) ELISA Kit

(Do not mix reagents of different batches and different product numbers in the kit, otherwise t he kit will not work properly)

Catalogue No.: EH20041

Size: 96T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of HBeAg in human serum or plasma.

Storage: 2-8°C

Expiry Date: see kit label

Principle: Sandwich

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	12 × 8	2-8℃/-20℃
HbeAg Positive Control	1ml×1	2-8℃
HbeAg Negative Control	1ml×1	2-8℃
Biotin- HBeAb (Concentrated, 100X)	120ul×1	2-8°C(Avoid Direct Light)
Antibody Dilution Buffer	10ml×1	2-8℃
HRP-Streptavidin (Ready to use)	10ml×1	2-8°C(Avoid Direct Light)
Sample dilution buffer	20ml×1	2-8℃
TMB substrate	10ml×1	2-8℃(Avoid Direct Light)
Stop solution	10ml×1	2-8℃
Wash buffer (25X)	30ml×1	2-8℃
Plate Sealer	3pieces	
Product Description	1 copy	

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Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. HBeAb was pre-coated onto 96-well plates. The test samples were added to the wells. Then added Biotin conjugated HBeAb, if there were any HBeAg in the samples, it would form a HBeAb- HBeAg - Biotin - HBeAb complex. After incubation, the unbound ingredients were washed away and HRP-Streptavidin was added. Wash off unbound ingredients after incubation, TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

Precautions

1. After opening and before using, keep plate dry.
2. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
3. Storage TMB reagents avoid light.
4. Washing process is very important, not fully wash easily cause a false positive and high background.
5. Duplicate well assay is recommended for both standard and sample testing.
6. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
7. Don't reuse tips and tubes to avoid cross contamination.
8. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manuf acturers.
9. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required but Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

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Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (Note: set the height of the needles; be sure the fluid can be sipped up completely)

Sample Collection and Storage

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

- **Serum:** Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.
- **Plasma:** Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

Note: Samples used within 5 days can be stored at 2-8°C; otherwise, they must be stored at -20°C or -80°C or liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test.

Wash Buffer Preparation:

Dilute 30mL of Concentrated Wash Buffer to 750mL of Wash Buffer with deionized or distilled water.

Preparation of Biotin-labeled Antibody Working Solution

The working solution should be prepared within 30min before the assay and can't be stored for a long time.

1.1. Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)

1.2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated biotin-labeled antibody to the bottom of tube.

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1.3. Dilute the biotinylated detection antibody with antibody dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated biotin-labeled antibody into 990ul antibody dilution buffer.)

Assay Procedure

1. Label the sample wells, 2 Negative Control wells, 2 Positive Control wells and 1 blank well.

2. Add 50μL sample dilution buffer to each well.

3. Add 50μL sample, Negative Control and Positive Control to the appropriate wells (except the blank well) and gently tap the plate to ensure thorough mixing.

4. Seal the plate with a cover and incubate at 37°C for 60 min.

5. Remove the cover, and wash the plate twice without immersion.

6. Add 100μL Biotin- HBeAb to each well. Seal the plate with a cover and incubate at 37°C for 30 min.

7. Wash the plate three times and immerse for 1min each time.

8. Add 100μL HRP-Streptavidin to each well. Seal the plate with a cover and incubate at 37°C for 30 min.

9. Remove the cover, and wash plate 5 times with Wash buffer, and let the wash buffer stay in the wells for 1 minute each time.

10. Add 90μl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark for 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

11. Keep the liquid in the well after staining. Add 50μl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

12. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)

Data Analysis

Calculation of the Cutoff Value

Cutoff Value = NCx x 2.1

NCx: Mean Absorbance of Negative Control. When NCx<0.05, Calculate as 0.05.

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Calculation of Results

Sample with absorbance values ≤ Cutoff Value is NON-REACTIVE and are considered NEGATIVE for HBeAg.

Sample with absorbance values > Cutoff Value are considered POSITIVE for HBeAg.

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