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Human HBeAg (hepatitis B virus E Antigen) ELISA Kit

Cat #: orb2816942 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Application	This kit allows for the qualitative determination of HBeAg in human serum or plasma.		
Reactivity	Human	Detection Method	Qualitative
Sample Types	Serum, Plasma, Cell Culture Supernatant, cell, tissue lysate and Other liquid samples		
Storage	2-8°C (for sealed box), please do not freeze!		

Assay Principle

This kit is based on a sandwich enzyme-linked immunosorbent assay (ELISA) technology. HBeAb is pre-coated onto 96-well plates. Test samples are added to the wells, followed by the addition of biotin-conjugated HBeAb. If HBeAg is present in the samples, a HBeAb–HBeAg–biotin–HBeAb complex is formed. After incubation, unbound components are removed by washing, and HRP–streptavidin is added. Following a second incubation and wash step, TMB substrate is added to visualize the HRP enzymatic reaction. HRP catalyzes the formation of a blue product, which turns yellow after the addition of an acidic stop solution. The optical density (OD) is measured at 450 nm using a suitable microplate reader, with a reference wavelength of 650 nm.

Kit Components

The sealed kit can be stored at 2-8 ℃. The storage condition for opened kit is specified in the table below:

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	

Materials Required but Not Supplied

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

Washing

Manual Washing:

Discard the solution from the plate without touching the side walls. Tap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350 μL of wash buffer and soak for 1–2 minutes. Then aspirate the contents and tap the plate again on absorbent filter paper or other absorbent material.

Automatic Washing:

Aspirate all wells, then wash the plate with 350 μL of wash buffer. After the final wash, invert the plate and tap it on absorbent filter paper or other absorbent material. It is recommended to set the washer for a 1-minute soak.

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Note: Adjust the height of the needles to ensure complete aspiration of liquid.

Sample Preparation

Sample Collection and Storage

Isolate test samples as soon as possible after collection and analyze immediately (within 2 hours). Alternatively, aliquot and store at -20°C for long-term storage. Avoid repeated freeze–thaw cycles.

- **Serum:** Allow blood to clot at room temperature (approximately 1 hour). Centrifuge at ~1000 × g for 15 minutes. Analyze the serum immediately or aliquot and store at -20°C.
- **Plasma:** Collect plasma using heparin or EDTA as the anticoagulant. Centrifuge at 1500 × g for 15 minutes at 2–8°C within 30 minutes of collection. To eliminate platelet effects, an additional centrifugation step at 10,000 × g for 10 minutes at 2–8°C is recommended. Analyze immediately or aliquot and store at -20°C.

Note: Samples to be used within 5 days may be stored at 2–8°C. For longer storage, keep samples at -20°C, -80°C, or in liquid nitrogen to avoid loss of biological activity and contamination. Avoid repeated freeze–thaw cycles. **Hemolytic samples are not suitable for this assay.**

Reagent Preparation

Wash Buffer

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

Biotin-labeled Antibody Working Solution

The working solution should be prepared within 30 minutes prior to the assay and should not be stored for extended periods.

1. Calculate the total required volume of working solution: 100 µL per well × number of wells. It is recommended to prepare an additional 100–200 µL.

2. Centrifuge the tube at 1000 × g for 1 minute at low speed to collect the concentrated biotin-labeled antibody at the bottom of the tube.

3. Dilute the biotinylated detection antibody 1:99 with antibody dilution buffer and mix thoroughly.

(e.g., add 10 µL of concentrated biotin-labeled antibody to 990 µL of antibody dilution buffer.)

Assay Procedure

1. Label the sample wells, two Negative Control wells, two Positive Control wells, and one blank well.
2. Add 50 µL of sample dilution buffer to each well.
3. Add 50 µL of 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 and incubate at 37°C for 60 minutes.
5. Remove the cover and wash the plate twice without soaking.
6. Add 100 µL of Biotin–HBeAb to each well. Seal the plate and incubate at 37°C for 30 minutes.
7. Wash the plate three times, soaking for 1 minute each time.
8. Add 100 µL of HRP–streptavidin to each well. Seal the plate and incubate at 37°C for 30 minutes.

9. Remove the cover and wash the plate five times with wash buffer, allowing the buffer to remain in the wells for 1 minute each time.
10. Add 90 µL of TMB substrate to each well. Cover the plate and incubate at 37°C in the dark for 15 minutes. A blue color should develop in the Positive Control wells, while the Negative Control wells should show no obvious color.
11. Without removing the liquid, add 50 µL of stop solution to each well and mix thoroughly. The color will immediately change to yellow.
12. Measure the optical density (OD) at 450 nm using a microplate reader immediately after adding the stop solution. (Use the blank well to set zero.)

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.

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.

Precautions

1. After opening and before use, keep the plate dry.
2. Before using the kit, centrifuge the tubes to bring all components to the bottom.
3. Store TMB reagent protected from light.
4. The washing process is critical; insufficient washing may result in false positives and high background.
5. Duplicate wells are recommended for both standards and samples.
6. Do not allow the microplate to dry during the assay, as this may inactivate active components on the plate.
7. Do not reuse tips or tubes to avoid cross-contamination.
8. Do not mix reagents from different kits from our company or from other manufacturers.
9. To ensure accurate results, ensure proper adhesion of the plate sealers during incubation steps.