

Human HBeAg ELISA Kit

Cat#: orb564674 (ELISA Manual)

Catalogue No.: orb564674

Size: 96T

Reactivity: Human

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

Storage: 2-8°C for 6 months.

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°C/-20°C
HBeAg Positive Control	1.0ml	2-8°C
HBeAg Negative Control	1.0ml	2-8°C
HRP- HBeAb	7ml	2-8°C
TMB substrate A	7ml	2-8°C(shading light)
TMB substrate B	7ml	2-8°C(shading light)
Stop solution	7ml	2-8°C
Wash buffer (20X)	25ml	2-8°C
Plate Sealer	3pieces	
Product Description	1 copy	

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 HRP conjugated HBeAb, if there were any HBeAg in the samples, it would form a HBeAb- HBeAg - HRP- HBeAb complex. 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. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.

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

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 $1000 \times g$ 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 $2-8^{\circ}\text{C}$ at $1500 \times g$ within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at $2-8^{\circ}\text{C}$ at $10000 \times g$. Analyze immediately or aliquot and store frozen at -20°C .

Note: Samples to be used within 5 days may be stored at $2-8^{\circ}\text{C}$, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Wash Buffer Preparation:

Dilute 25mL of Concentrated Wash Buffer to 500mL of Wash Buffer with deionized or distilled water.

Assay Procedure

1. Label the sample wells, 2 Negative Control wells, 2 Positive Control wells and 1 blank well.
2. Add $50\mu\text{L}$ sample, Negative Control and Positive Control to the appropriate wells, except the blank well.
3. Add $50\mu\text{L}$ HRP- HBeAb to each well, except the blank well. Gently tap the plate to ensure thorough mixing.
4. Seal the plate with a cover and incubate at 37°C for 60 min, and balance it at room temperature for 5 min.
5. 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.
6. Add $50\mu\text{L}$ of TMB substrate A and $50\mu\text{L}$ of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. 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.
7. Add $50\mu\text{L}$ of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
8. 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

If Negative control >0.1 , or Positive control ≤ 0.4 , it regarded as the test is Invalid.

Calculation of the Cutoff Value

Cutoff Value = $\text{NCx} \times 2.1$

NCx: Mean Absorbance of Negative Control. When $\text{NCx} < 0.05$, Calculate as 0.05.

Calculation of Results

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

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