

## Human Pre-S1 ELISA Kit

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

**Catalogue No.:** EH4111

**Size:** 96T

**Reactivity:** Human

**Application:** This immunoassay kit allows for the qualitative determination of HBV Pre-S1-antigen 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°C/-20°C
HBV Pre-S1 Positive Control	1ml	2-8°C
HBV Pre-S1 Negative Control	1ml	2-8°C
HRP-HBsAg-Antibody	7ml	2-8°C(Avoid Direct Light)
TMB Substrate A	7ml	2-8°C(Avoid Direct Light)
TMB Substrate B	7ml	2-8°C(Avoid Direct Light)
Stop Solution	7ml	2-8°C
Wash Buffer (25X)	25ml	2-8°C
Plate Sealer	5pieces	
Product Description	1 copy	

## Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-HBV Pre-S1-Antibody was pre-coated onto 96-well plates. The test samples were added to the wells. Then added HBsAg-Antibody, if there were any HBV Pre-S1-antigen in the samples, it would form a Anti-HBV Pre-S1-Antibody- HBV Pre-S1-antigen- HRP-HBsAg-Antibody 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. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. 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

## 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 × g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 × g. Analyze immediately or aliquot and store frozen at -20°C.

#### **Note:**

1. Human serum or plasma samples are used, and the plasma samples are commonly used anticoagulants (heparin, sodium citrate, EDTA) It does not affect the test results.
2. If serum and plasma samples are tested within 7 days after collection, they can be stored at 2~8 °C; More than 7 days must be set to -20 °C The following are cryopreserved. Cryopreserved samples should be completely thawed, rewarmed, and mixed before use. Avoid repeating Freeze-thaw, otherwise the test results may be affected.

### 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 50µL sample, Negative Control and Positive Control to the appropriate wells (except the blank well).
3. Add 50µL HRP-HBsAg Antibody to each well, except 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.
5. Remove the cover, and wash plate 5 times with Wash buffer, and let the wash buffer stay in the wells for 0.5-1 minute each time.
6. Add 50µl of TMB substrate A and 50µl 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 50µl 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)(Readings are taken within 30 minutes of termination of reaction)

## Data Analysis

Calculation of the Cutoff Value

Cutoff Value(C.O.) = NCx x 4

NCx: When the negative mean A value is less than or equal 0.05, it is calculated as 0.05. When the negative mean A value is greater than 0.05, it is calculated according to the actual value.

## Calculation of Results

The sample had an OD value of S/C.O.  $\geq 1$  and was positive for HBV pre-S1 (Pre S1).

The sample had an OD value of S/C.O.  $< 1$  and was negative for HBV pre-S1 (Pre S1).

When the OD value of the positive control is  $\leq 0.4$ , the experiment is invalid and the test should be retested.

## Limitations of the test method

1. This method is only suitable for the detection of human serum (or plasma) samples.
2. This method only qualitatively detects the HBV pre-S1 antigen in human serum (or plasma), and cannot be used as a quantitative test agent use.