Technical Manual

Human HBeAg (hepatitis B virus E Antigen) ELISA Kit

- Catalogue Code: HUFI04751
- Sandwich ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

HBeAg

Detection method:

Sandwich

Sample Type:

Serum, Plasma and other biological fluids

Reactivity:

Human

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

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 catalysed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The optical density of developed colour is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays including:

No.	Component	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	12 x 8	2-8°C/-20°C
2	HBeAg Positive Control	1ml x 1	2-8°C
3	HBeAg Negative Control	1ml x 1	2-8°C
4	HRP-HBeAb	7ml x1	2-8°C
5	TMB substrate A	7ml x 1	2-8°C (Avoid Direct Light)
6	TMB substrate B	7ml x1	2-8°C (Avoid Direct Light)
7	Stop solution	7ml x1	2-8°C
8	Wash buffer (20X)	25ml x 1	2-8°C
9	Plate Sealer	3	
10	Manual	1	

Additional materials required:

1. 37°C incubator
2. Plate Reader with 450nm filter
3. Precision pipettes and disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

Precautions:

1. To identify the concentration of your target, a 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, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross contamination.

9. Avoid using the reagents from different batches together.

5. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated 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: Over haemolyzed samples are not suitable for use with this kit.

Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Hemolyzed samples are not suitable for this assay.

6. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

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

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: Matrix components in the sample will affect test results, samples need to be diluted at least ½ with Sample Dilution Buffer before testing.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use.

1. Wash Buffer:

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

7. Assay Procedure

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

2. Add 50µL Negative Controls and Positive Controls to each well (except blank well).

3. Add 50µL HRP-HBeAb to each well, except blank well

4. Seal the plate with a cover and incubate at 37°C for 60 min. Followed by a rest at Room Temperature for 5 mins.

5. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 1- 2 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 colour.

7. Add 50µl of Stop solution into each well and mix thoroughly. The colour 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).

8. Data Analysis

Cut-off Value = NCx x 2.1

NCx: Mean Absorbance of Negative Control (When the negative mean A value is less than 0.05, it is calculated as 0.05 when the negative mean A value is greater than or equal to 0.05, it is calculated according to the actual value.). If Negative control >0.1, or Positive control ≤ 0.4, it regarded as the test is Invalid.

2. Determination of results

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

Notes

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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