Human EB-EA-IgG (Epstein-barr virus antigen Early antigen-Immunoglobulin G) 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.: EH4739

Size: 96T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of EB-EA-IgG in human serum.

Storage: 2-8℃

Expiry Date: see kit label

Principle: Indirect

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	12 × 8	2-8℃/-20℃
EB-EA-IgG Positive Control	1ml×1	2-8℃
EB-EA-IgG Negative Control	1ml×1	2-8℃
Sample dilution buffer	12ml×1	2-8℃
HRP- Conjugates	12ml×1	2-8℃(Avoid Direct Light)
TMB substrate A	6ml×1	2-8℃(Avoid Direct Light)
TMB substrate B	6ml×1	2-8℃(Avoid Direct Light)
Stop solution	6ml×1	2-8℃
Wash buffer (20X)	50ml×1	2-8℃
Plate Sealer	3pieces	
Product Description	1 copy	

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Principle of the Assay

This kit was based on indirect ELISA. EB-EA was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP- Conjugates, if there were any EB-EA-IgG in the samples, it would form a EB-EA - EB-EA-IgG- HRP- Conjugates 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 for Use

1.After opening and before using, keep plate dry.

2. Before using the Kit, balance the reagents at room temperature at least 30 mins.

3. Storage TMB reagents avoid light.

4. Washing process is very important, not fully wash easily cause a false positive.

5. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.

6. Don't reuse tips and tubes to avoid cross contamination.

7. Avoid using the reagents from different batches together.

Material Required But Not Supplied

1.Microplate reader (wavelength: 450nm)

2.37℃ 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

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers 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. Repeat this procedure two more times for a total of THREE washes.

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Automated Washing

Aspirate all wells, then wash plate THREE times 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 be set for a soaking time of 1 minute.

Sample Collection and Storage

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

- Serum: Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.

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 50ml concentrated wash buffer to 1000ml wash buffer with deionized or distilled water.

Assay Procedure

Put the kit at room temperature for 30 minutes before use.

1. Take out the wells that need to be used, and store the remaining wells in the sealed bag.Label the sample wells, 3 Negative Controls, 1 Positive Controls and 1 blank wells.
2. Add 100µL Negative Controls and Positive Controls to each wells(except blank well).
3. Add 100µL sample dilution buffer to each sample well, then add 10µL sample to sample well and gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 30 min.
4. 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.
5. Add 100 µL HRP- Conjugates to each well, except blank well
6. Seal the plate with a cover and incubate at 37℃ for 30 min.
7. 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.

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8. Add 50 µl of TMB substrate A and 50 µl of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37℃ in dark within 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

9. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

10. 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

- **1. Calculation of the Cutoff Value**

Cutoff Value = **NCx**+0.1

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.)

- 2. **Determination of results**

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

- 3. **Quality control**

The blank well (only adding TMB and Stop solution) should not be greater than 0.08.

The positive control (PC) A value was greater than 0.80.

The negative mean A value was less than 0.1.

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