Human Anti-HPV16 (Papillomavirus 16) IgG 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.: EH5623

Size: 96T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of anti-HPV16 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℃
Anti-HPV16 IgG Positive Control (Ready-to-use)	1ml×1	2-8℃
Anti-HPV16 IgG Negative Control (Ready-to-use)	1ml×1	2-8℃
Sample dilution buffer	20ml×1	2-8℃
HRP-Anti-Human IgG Antibody	5ml×1	2-8°C (Avoid Direct Light)
TMB Substrate	5ml×1	2-8°C (Avoid Direct Light)
Stop solution	5ml×1	2-8℃
Wash Buffer (25 x concentrate)	30ml×1	2-8℃
Plate Sealer	5pieces	2-8℃
Product Description	1 copy	

Principle of the Assay

This kit was based on indirect enzyme-linked immune-sorbent assay technology. HPV16-Ag 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- Anti-Human IgG Antibody, if there were any anti-HPV16 IgG in the samples, it would form a HPV16-Ag- anti-HPV16 IgG- HRP- Anti-Human IgG Antibody complex.The substrate TMB was added for color development, and the presence or absence of anti-HPV16 IgG antibody was determined according to OD value after colorimetry on the enzyme label.

Precautions for Use

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 manuf acturers.
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℃ 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.

Automated Washing

Aspirate all wells, 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 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.

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

Wash Buffer Preparation:

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

Assay Procedure

Remove the kit from the refrigerated environment and let it equilibrate at room temperature for 30 minutes before use

1. Label the sample wells, 3 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 sample dilution buffer to sample wells and then add 5µL sample serum. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37℃ for 30 min.

4. Remove the cover, and wash plate 3 times with Wash buffer and let the wash buffer stay in the wells for 1 minute each time. Wash the board 3 times in a row and pat dry the last time.

5. Add 50µL HRP-Anti-Human IgG Antibody 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 1 minute each time. Wash the board 5 times in a row and pat dry the last time.

8. Add 50 µl of TMB substrate 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)(Readings are taken within 30 minutes of termination of reaction)

Data Analysis

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

Cutoff Value =NCx× 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.)

- 2. **Determination of results**

Sample with absorbance values < Cutoff Value are NON-REACTIVE and are considered NEGATIVE for anti-HPV16 IgG.

Sample with absorbance values ≥ Cutoff Value are considered POSITIVE for anti-HPV16 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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