Human HPV16 L1 Ab ELISA Kit

Catalog No.: BEK1257

Size: 96 T

Storage and Expiration: Store at 2-8℃ for 6 months, at -20℃ for 12 months. Store HRP conjugated antibody at 4℃ for one year.

Application: For qualitative detection of HPV16 L1 Ab in Human serum, plasma or cell culture supernatant.

Introduction

Papillomaviridae is an ancient taxonomic family of non-enveloped DNA viruses, collectively known as papillomaviruses. Antibodies to HPV16 proteins are associated with increased risk for HPVOPC. Among patients with OPC, HPV16 Abs are associated with tumor HPV status, in particular among HPV positive patients with no or little smoking history. The amino (N) terminus of the human papillomavirus (HPV) minor capsid protein L2 can induce low-titer, cross-neutralizing antibodies. Immunization with HPV16 L1-HPV16 L2 (chimera 17-36) VLP in adjuvant applicable for human use induces broad-spectrum neutralizing antibodies against HPV types evolutionarily divergent to HPV16 and thus may protect against infection with mucosal high-risk, low-risk, and beta HPV types and associated disease.

Principle of the Assay

This kit was based on indirect enzyme-linked immune-sorbent assay technology. The recombinant HPV16 L1 antigens were pre-coated onto 96-well plates. The test samples were added to the wells and incubated subsequently, the HPV16 L1 antibodies in the test samples combined to the pre-coated antigens specifically, then followed by washing with wash buffer to remove unspecific components. And the HRP conjugated antibodies were added and incubated to bind to human antibodies in plate, the unbound conjugates were washed away with wash buffer. TMB substrate were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. Read the O.D. absorbance at 450 nm in a microplate reader.

Kit components

1. One 96-well plate pre-coated with Human HPV16 L1 antigen
2. Negative control: 0.5 ml
3. Positive control: 0.5 ml
4. Sample diluent buffer: 30 ml
5. HRP conjugated anti-Human antibody (Concentrated): 130 µl. Dilution: 1:100 **(Store at 4℃, do NOT store it at -20℃).**
6. Antibody diluent buffer: 12 ml
7. TMB substrate: 10 ml
8. Stop solution: 10 ml
9. Wash buffer: 30 ml (25x). Dilution: 1:25

Material Required But Not Provided

1. 37℃ incubator
2. Microplate reader (wavelength: 450nm)
3. Precise pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml of Eppendorf tubes
7. Absorbent filter papers
8. Plastic or glass container with volume of above 1L

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Protocol

- **Preparation of sample and reagents**

1. Sample

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 at room temperature for 10-20 min, then, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. If precipitation appeared, centrifuge again.
- ✧ **Plasma:** Collect plasma using EDTA or citrate plasma as an anticoagulant, and mix for 10-20 min, centrifuge at the speed of 2000-3000 r.p.m. for 20 min of collection. If precipitation appeared, centrifuge again.

Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle.
2. NaN_3 cannot be used as test sample preservative, since it is the inhibitor for HRP.
3. After collecting samples, analyze immediately or aliquot and store frozen at -20°C. Avoid repeated freeze-thaw cycles.

2. Wash buffer

Dilute concentrated Wash buffer (Kit Component 9) 25-fold (1:25) with distilled water (i.e. add 20 ml of concentrated wash buffer into 480 ml of distilled water).

3. Preparation of HRP conjugated anti-Human antibody (Kit Component 5) working solution: the HRP conjugated antibody should be used within 30 min after diluting.

- ✧ Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
- ✧ Dilute the HRP conjugated anti-Human antibody (Kit Component 5) with Antibody diluent buffer (Kit Component 6) at 1:100 and mix thoroughly. i.e. Add 1 μl of HRP conjugated anti-Human antibody into 99 μl of Antibody diluent buffer.

- **Assay procedure**

1. Equilibrate kit components for 15-30 min at room temperature.
2. Set test sample, negative control (2 wells), positive control (2 wells), blank control (1 well) wells on the pre-coated plate respectively, and then, record their positions. Do not add sample and HRP conjugated antibody into the blank control well.
3. Add 100 μl of Negative control (Kit component 2) and Positive control (Kit component 3) into negative control and positive control wells respectively (NC1, NC2 & PC1, PC2). For test sample wells, add 100 μl of properly diluted sample (serum, plasma or cell culture supernatant) into test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37℃ for 90 min.
5. Remove the sealer, and wash plate using one of the following methods:
   Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers. Fill each well completely with Wash Buffer (1×) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers. Repeat this procedure four more times for a **total of THREE washes**.
   Automated Washing: Aspirate all wells, then wash plates **THREE times** using Wash Buffer (1×).

After the final wash, invert plate, and clap the plate on absorbent filter papers until no moisture remained. It is recommended that the washer be set for a soaking time of 10 seconds or shaking.

6. Add 100 µl of HRP conjugated anti-Human antibody work solution into each well (except blank control well).
7. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37℃ for 60 min.
8. Remove the sealer, and wash the plate for **FIVE TIMES**. (See Step 5)
9. Add 100 µl of TMB substrate (Kit Component 7), cover the plate and incubate at 37℃ in dark within 10 min. (**Note:** The color development on the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue color.) The shades of blue can be seen in the wells.
10. Add 100 µl of Stop solution (Kit Component 8) into each well and mix thoroughly. The color changes into yellow immediately.
11. Read the O.D. absorbance at 450nm in a microplate reader within 15 min after adding the stop solution.

- **Results analysis**

1. Calculations

$O.D._{450}$ of mean positive control: PC = ($O.D._{450}$ of PC1 + $O.D._{450}$ of PC2)/2

$O.D._{450}$ of mean negative control: NC = ($O.D._{450}$ of NC1 + $O.D._{450}$ of NC2)/2

2. Interpretation of results

If ($O.D._{450}$ of samples - O.D. of blank) / (NC - O.D. of blank) < 2.1, the test samples are negative;

If ($O.D._{450}$ of samples - O.D. of blank) / (NC - O.D. of blank) ≥ 2.1, the test samples are positive.

Precautions

1. All reagents should be considered as potentially hazardous. It is recommend that this kit is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

2. Store HRP conjugated anti-Human antibody (concentrated) (Kit Component 5) at 4℃, do NOT store it at -20℃.

3. Do not use expired components or mix components from different lots or suppliers.
4. Avoid contact of skin or mucous membranes with kit reagents or specimens.
5. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
6. Avoid contact of substrate solution with oxidizing agents and metal.
7. Avoid splashing or generation of aerosols.
8. To avoid microbial contamination or cross-contamination of reagents or specimens, it is recommended to use the clean and separate pipette tip for each.
9. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent. Exposure to acid inactivates the conjugate.
10. Distilled or deionized water must be used for reagent preparation.
11. Substrate solution must be equilibrated at room temperature prior to use.

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Reference

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