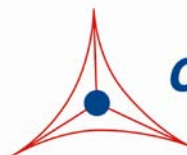

Product Manual

HPV16 E7 Oncoprotein ELISA Kit

Catalog Numbers

VPK- 5045	96 assays
VPK- 5045- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Human papillomavirus (HPV) is a large group of more than 150 related DNA viruses. HPV is named for the warts (papillomas) some types can cause and is spread through direct skin-to-skin contact. Although most HPV infections cause no symptoms and resolve spontaneously, some persist and result in warts or precancerous lesions. Of the more than 150 types, over 40 types are known to be transmitted through sexual contact and make it the most commonly sexually transmitted infection (STI).

HPV-induced cancers arise when viral sequences are integrated into the DNA of host cells. Some genes carried by the HPV virus, such as genes E6 and E7, act as oncogenes that promote tumor growth and malignant transformation. Roughly 12 HPV types (including 16, 18, 31, and 45) are classified as high-risk for being linked to malignancies. Nearly all cases of cervical cancer are associated with HPV infection, with two types present in 70% of cases: HPV16 and HPV18. Previous studies suggest that high-risk E7 oncoproteins are necessary for this cancer, by inactivating cell-cycle regulatory proteins. The ability to monitor these E7 levels may be a useful tool in cervical cancer screening and detection.

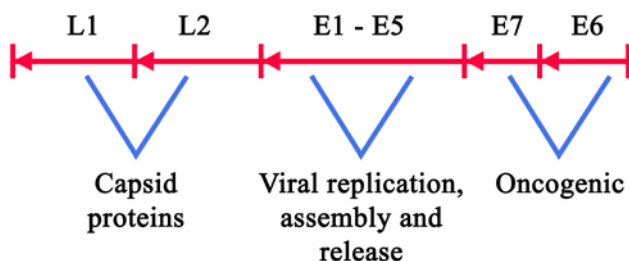


Figure 1: Schematic Presentation of the HPV Genome

Cell Biolabs' HPV16 E7 Oncoprotein ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the Human Papillomavirus Type 16 E7 protein. The kit has detection sensitivity limit of 78 pg/mL HPV16 E7. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and HPV16 E7 samples.

Assay Principle

An anti-HPV coating antibody is adsorbed onto a microtiter plate. HPV16 E7 protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-HPV16 E7 antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-HPV16 E7 antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of HPV16 E7 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from recombinant HPV16 E7 and sample concentration is then determined.

Related Products

1. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV-1 p24 ELISA)
2. VPK-150: QuickTiter™ HBcAg ELISA Kit

3. VPK-151: QuickTiter™ HCcAg ELISA Kit
4. VPK-5003: QuickTiter™ HBeAg ELISA Kit
5. VPK-5004: QuickTiter™ HBsAg ELISA Kit
6. VPK-5046: HPV18 E7 Oncoprotein ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-HPV Antibody Coated Plate (Part No. 50451B): One strip well 96-well plate.
2. Biotinylated Anti-HPV16 E7 Antibody (1000X) (Part No. 50452D): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Recombinant HPV16 E7 Standard (Part No. 50453D): One 100 µL vial of 5 µg/mL rHPV16 E7.

Materials Not Supplied

1. HPV Sample: cell or tissue lysate, cervical smear
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store recombinant HPV16 E7 Standard at -20°C and avoid freeze/thaw. Store all other components at 4°C.

Safety Considerations

Remember that your samples contain infectious viruses; you must follow the recommended NIH guidelines for all materials. Handle as a potentially hazardous substance.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-HPV16 E7 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-HPV16 E7 Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of Recombinant HPV16 E7 Standard in the concentration range of 5 ng/mL – 0.078 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	5 µg/mL Recombinant HPV16 E7 Standard (µL)	Assay Diluent (µL)	HPV16 E7 (ng/mL)
1	4	3996	5
2	500 of Tube #1	500	2.5
3	500 of Tube #2	500	1.25
4	500 of Tube #3	500	0.625
5	500 of Tube #4	500	0.313
6	500 of Tube #5	500	0.156
7	500 of Tube #6	500	0.078
8	0	500	0

Table 1. Preparation of HPV16 E7 Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of HPV16 E7 sample or standard to the Anti-HPV Antibody Coated Plate. Each HPV16 E7 sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-HPV16 E7 Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 μL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.
Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. Stop the enzyme reaction by adding 100 μL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical HPV16 E7 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

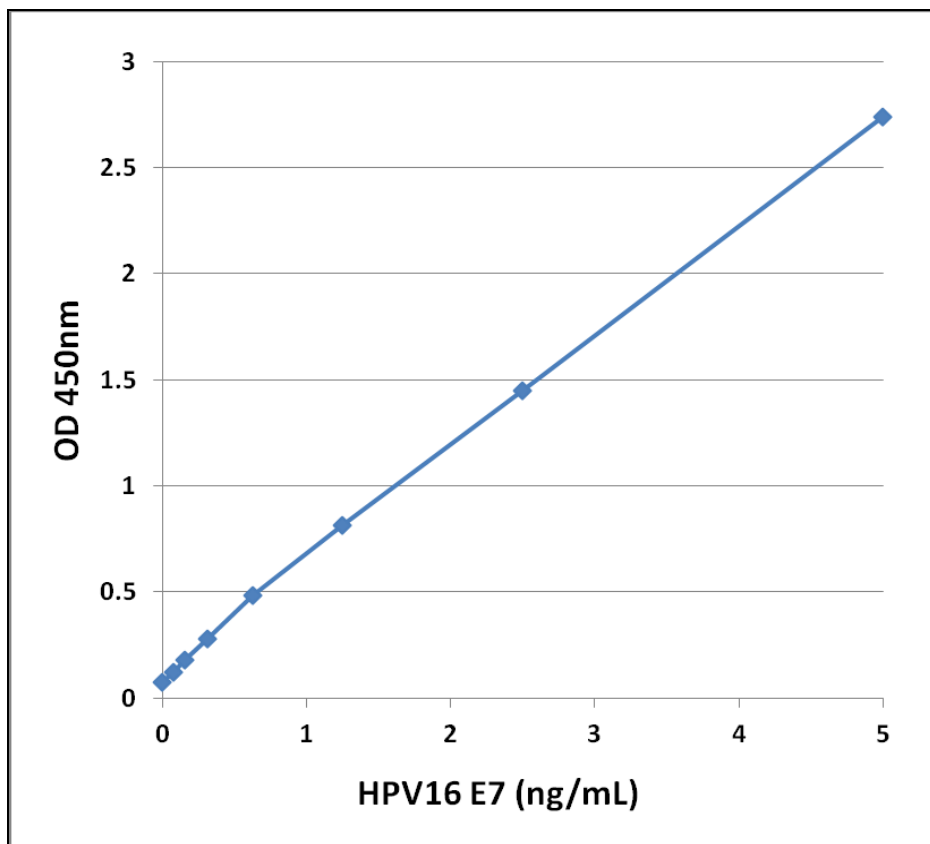


Figure 2: HPV16 E7 ELISA Standard Curve

References

1. Griffin, H., R. Elston, D. Jackson, K. Ansell, M. Coleman, G. Winter, and J. Doorbar (2006) *J. Mol. Biol.* **355**:360-378.
2. Kong, Q., W. Wang, and P. Li (2015) *Int. J. Clin. Exp. Pathol.* **8**:15808-15813.
3. Van Doorslaer, K., L. Reimers, Y. Studentsov, M. Einstein, and R. Burk (2010) *Gynecol. Oncol.* **116**:208-212.
4. Williams, V., M. Filippova, V. Filippov, K. Payne, and P. Duerksen-Hughes (2014) *J. Virol.* **88**:6751-6761.

Warranty

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