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User's Manual

Human HPV 16 E7 Oncoprotein ELISA Kit

REF **DEIASL171**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Human HPV16 E7 Oncoprotein ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the Human Papillomavirus Type 16 E7 protein from cell lysates, tissue lysates, cervical smears, plasma and serum. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and HPV16 E7 samples.

General Description

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.

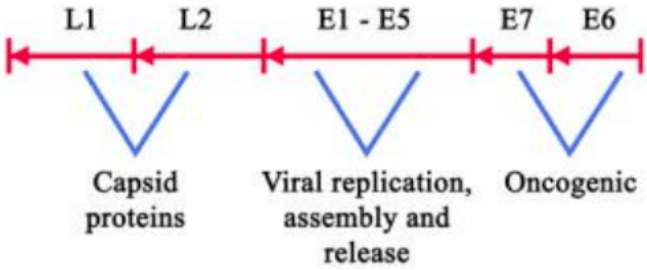


Figure 1: Schematic Presentation of the HPV Genome

Principles of Testing

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.

Reagents And Materials Provided

1. Anti-HPV Antibody Coated Plate: One strip well 96-well plate.
2. 100× Anti-HPV16 E7 Biotin: One 120 µL vial.
3. 100× Streptavidin-enzyme Conjugate: One 120 µL vial.
4. Recombinant HPV16 E7 Standard (500 ng): lyophilized powder.
5. Assay Diluent: One 50 mL bottle.
6. 20× Wash Buffer: One 50 mL bottle.
7. Substrate Solution: Two 6 mL bottles.
8. Stop Solution: One 7 mL bottle.

Materials Required But Not Supplied

1. HPV Sample: cell or tissue lysate, cervical smear, plasma, serum.
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).
6. Ultrapure water.

Storage

After you receive the kit, all the components should be stored in the refrigerator (4-8°C) also up to 1 year. Long term storage, improper storage conditions and large temperature fluctuation cycles may cause precipitates in the TMB solution. These precipitates should not affect the assay noticeably. Nevertheless, if you observe such precipitates, we recommend to avoid them by allowing them to sink to the bottom.

Reagent Preparation

1× Wash Buffer:

Dilute the 20× Wash Buffer to 1× with deionized water. Stir to homogeneity.

1× Anti-HPV16 E7 Biotin and Streptavidin-Enzyme Conjugate:

Immediately before use dilute the Anti-HPV16 E7 Biotin 1:100 and the Streptavidin-Enzyme Conjugate 1:100 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. The Recombinant HPV16 E7 Standard (500 ng) is provided as a lyophilized stock. Reconstitute it with 1 mL ultrapure water as a 500ng/ml stock solution. The reconstituted stock should be aliquoted and stored below -20°C.
2. Prepare a dilution series of Recombinant HPV16 E7 Standard in the concentration range of 0.078 ng/mL – 5 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Suggested Preparation of Standards			
	ng/ml	Range: 0.078 to 5 ng/ml	
Stock	500		
S1	5	Add 10µl Stock	+990 µl Diluent
S2	2.5	Add 250µl S1	+250 µl Diluent
S3	1.25	Add 250µl S2	+250 µl Diluent
S4	0.625	Add 250µl S3	+250 µl Diluent
S5	0.313	Add 250µl S4	+250 µl Diluent
S6	0.156	Add 250µl S5	+250 µl Diluent
S7	0.078	Add 250µl S6	+250 µl Diluent
S0	0.00		250µl Diluent

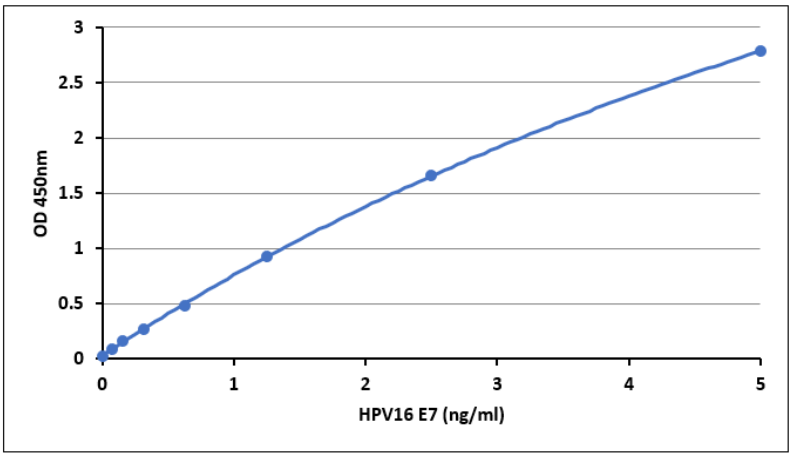
Table 1. Preparation of HPV16 E7 Standard

Assay Procedure

1. Before performing the assay, samples and assay kit should be brought to room temperature (about 30 minutes beforehand) and ensure the homogeneity of the solution.
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.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 300 µL 1× 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 1× Wash Buffer.
5. Add 100 µL of the diluted Anti-HPV16 E7 Biotin to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour.
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.
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 for 15 minutes.
12. Stop the enzyme reaction by adding 50 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.

Typical Standard Curve

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.



Detection Range

0.078-5ng/mL.

Sensitivity

The kit has detection sensitivity limit of 31 pg/mL HPV16 E7.

Precautions

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

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.