



User's Manual

Human HPV 18 E7 Oncoprotein ELISA Kit

REF DEIASL172

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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 HPV18 E7 Oncoprotein ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the Human Papillomavirus Type 18 E7 protein. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and HPV18 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.

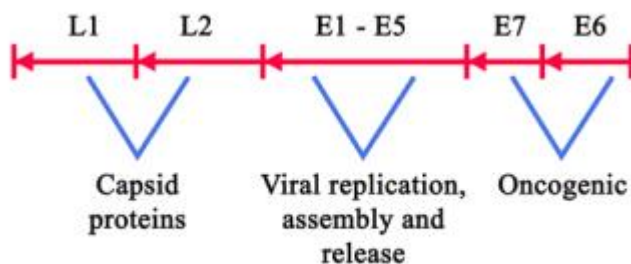


Figure 1: Schematic Presentation of the HPV Genome

Principles of Testing

An anti-HPV coating antibody is adsorbed onto a microtiter plate. HPV18 E7 protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-HPV18 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-HPV18 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 HPV18 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 HPV18 E7 and sample concentration is then determined.

Reagents And Materials Provided

Box 1 (shipped at room temperature)

1. Anti-HPV Antibody Coated Plate: One strip well 96-well plate.
2. Biotinylated Anti-HPV18 E7 Antibody (1000X): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (1000X): One 20 µL vial.
4. Assay Diluent: One 50 mL bottle.
5. 10X Wash Buffer: One 100 mL bottle.
6. Substrate Solution: One 12 mL amber bottle.
7. Stop Solution: One 12 mL bottle.

Box 2 (shipped on blue ice packs)

Recombinant HPV18 E7 Standard: One 100 µL vial of 5 µg/mL rHPV18 E7.

Materials Required But 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.

Reagent Preparation

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-HPV18 E7 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-HPV18 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 HPV18 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 HPV18 E7 Standard (µL)	Assay Diluent (µL)	HPV18 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 HPV18 E7 Standard

Assay Procedure

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 μ L of HPV18 E7 sample or standard to the Anti-HPV Antibody Coated Plate. Each HPV18 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 μ L 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 μ L of the diluted Biotinylated Anti-HPV18 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 μ L 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 μ L 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 μ L 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 HPV18 E7 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

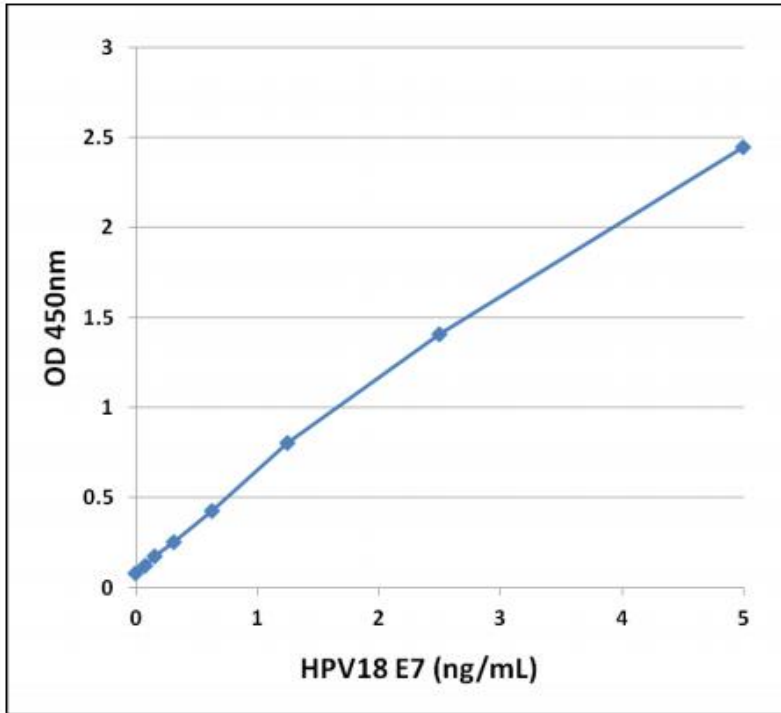


Figure 2: HPV18 E7 ELISA Standard Curve

Detection Range

0.078-5ng/mL

Sensitivity

The kit has detection sensitivity limit of 78 pg/mL HPV18 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.
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4. Williams, V., M. Filippova, V. Filippov, K. Payne, and P. Duerksen-Hughes (2014) *J. Virol.* 88:6751-6761.