

TechNotes



Clinical and

Tumor Markers



Infectious Diseases



Human papilloma virus (HPV)

uman papillomavirus (HPV) belongs to Papillomaviruses, a diverse group of DNA-based viruses that infect the skin and mucous membranes of humans and a variety of animals. Over 100 different human papillomavirus (HPV) types have been identified on the basis of difference in the virus genome nucleotide sequences (e.g. type 1, 2 etc.)

Today genital HPV infection is one of the most widespread sexually transmitted diseases. All HPVs are transmitted by skin-to-skin contact. A group of about 30-40 HPVs is typically transmitted through sexual contact and infect the anogenital region. Some sexually transmitted HPVs, types 6 and 11, may cause genital warts. However, other HPV types which may infect the genitals do not cause any noticeable signs of infection.

Persistent infection with a subset of about 13 so-called "high-risk" sexually transmitted HPVs, including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 — different from the ones that cause warts — may lead to the development of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), penile intraepithelial neoplasia (PIN), and/or anal intraepithelial neoplasia (AIN).

These are precancerous lesions and can progress to invasive cancer. HPV infection is a necessary factor in the development of nearly all cases of cervical cancer.

The HPV lifecycle begins from infection of epithelial tissues through micro-abrasions. At this point, the viral genome is transported to the nucleus and establishes itself at a copy number between 10-200 viral genomes per cell. A sophisticated transcriptional cascade then occurs as the host keratinocyte begins to divide and become increasingly differentiated in the upper layers of the epithelium. The viral oncogenes, E6 and E7, are thought to modify the cell cycle so as to make them amiable to the amplification of viral genome replication and consequent late gene expression. In the upper layers of the host epithelium, the late genes L1 and L2 are transcribed/translated and serve as structural proteins which encapsidate the amplified viral genomes.

HyTest offers a wide spectrum of monoclonal antibodies specific to oncoprotein E7 of "high-risk" HPV types 16 and 18. MAbs can be used in routine immunoassays (direct or indirect ELISA, sandwich immunodetection systems, Western blotting).

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks	
Human papillomavirus, type 16, oncoprotein E7	3HP16	716-325	IgG2a	EIA, WB	
		716-332	IgG1	EIA, WB, C/r with type 18	
		716-D1	IgG2a	EIA, WB, C/r with type 18	
Human papillomavirus, type 18, oncoprotein E7	3HP18	718-15	IgG1	EIA, WB, C/r with type 16	
		718-67	IgG2a	EIA, WB, C/r with type 16	

ANTIGENS

Product name	Cat. #	Purity	Source
Human papillomavirus L1 protein (HPVL1), type 16, recombinant	8HPV16	>90%	Recombinant
Human papillomavirus L1 protein (HPVL1), type 18, recombinant	8HPV18	>90%	Recombinant

Monoclonal antibodies specific to HPV, type 16, oncoprotein E7

E7 HPV type 16 immunodetection in ELISA

The best combination of monoclonal antibodies for E7 HPV type 16 sandwich ELISA were selected from several MAb combinations. The pairs were selected on the basis of MAb mapping data (maximal spatial determinant separation), sensitivity, specificity and kinetics characteristics.

Recommended pairs for sandwich ELISA are (capture - detection): 716-D1 - 716-332 (Fig. 1)

MAbs 716-D1, and 716-325 are equally suitable for capture of both HPV type 16 and 18.

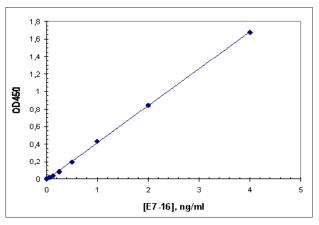


Figure 1. Calibration curves for E7 HPV type 16 sandwich immunoassays: 716-D1 - 716-332.

Coating: MAb 716-D1, 5 µg/ml, 0.1 M Carbonate buffer, pH 9.2 Detection: HRP-conjugated MAb 716-332, 1/50 000

Substrate: TMB

E7 HPV type 16 immunodetection in Western blotting

The results of MAb E7 HPV type 16 immunodetetion in Western blotting after antigen SDS-gel electrphoresis and its transfer onto nitrocellulose membrane are presented on Figure 2. Most of the tested MAbs recognize both monomer and dimer (most common in physiological media) forms of HPV type 16.

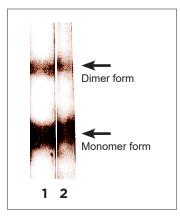


Figure 2. Detection of E7 HPV type 16 in Western blotting by different monoclonal antibodies after 15% SDS-PAAG electrophoresis. Strip 2: MAb 716-332 Strip 3: MAb 716-D1 E7 HPV type 16 quantity: 15.0 μg/strip.

Monoclonal antibodies specific to HPV, type 18, oncoprotein E7

E7 HPV type 18 immunodetection in Western blotting

The results of MAb E7 HPV type 18 immunodetection in Western blotting after antigen SDS-gel electrophoresis and its transfer onto nitrocellulose membrane are presented in Figure 3. As can be seen MAb 716-D1 has ability to recognize E7 oncoprotein of both HPV type 16 and 18 that makes them suitable as a capture antibody for determination of both types of HPV. MAb 718-67 was found to be able to recognize both monomer and dimer forms of HPV type 18 and can be recommended for an ELISA application.

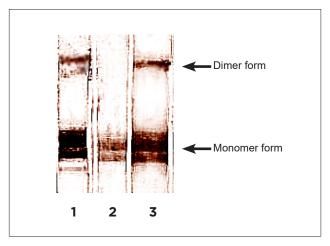


Figure 3. Detection of E7 HPV type 18 in Western blotting by different monoclonal antibodies after 15% SDS-PAAG electrophoresis:

Strip 1: MAb 716-D1 Strip 2: MAb 718-15

Strip 3: MAb 718-67

E7. HPV type 18 quantity: 15.0 μg/strip.

The main characteristics of all available antibodies are presented in Table 1.

Table 1. MAbs main characteristics. Study of cross-reactivity with HPV types 16 and 18 was done in indirect ELISA (coating 5 mg/ml of each antigen; MAbs 3 mg/ml.)

MAb		mmunogen 70 conjugated):	Cross reactivity with:		
HPV type		E7 oncoprotein fragment	E7, type 11	E7, type 16	E7, type 18
716-325	16	Whole molecule	0%	100%	0%
716-332	16	Whole molecule	0%	100%	43%
716-D1	16	Whole molecule	0%	100%	114%
718-15	18	Whole molecule	7%	43%	100%
718-67	18	Whole molecule	0%	9%	100%



