



Clinical and
Research Area

Tumor
Markers



Infectious
Diseases



Human papilloma virus (HPV)

Human 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. Approximately 20 million people around the world are currently infected with HPV. At least 50 percent of sexually active men and women acquire genital HPV infection at some point in their lives. By age 50, at least 80 percent of women will have acquired genital HPV infection. In accordance with WHO information, genital HPV infection was a reason of over 99% of cervical cancer cases, i.e. about 1.4 million women were affected worldwide and 239 000 of them died each year.

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 as well as of less oncogenic HPV type 11. MAbs can be used in routine immunoassays (direct or indirect ELISA, sandwich immunodetection systems, Western blotting). Some MAbs display high specificity to definite type of HPV while others can be used for determination of E7 proteins for all four types of viruses.

Monoclonal antibodies specific to HPV, type 11, oncoprotein E7

E7 HPV type 11 immunodetection in Western blotting

The results of MAb E7 HPV type 11 immunodetection in Western blotting after antigen SDS-gel electrophoresis and its transfer onto nitrocellulose membrane are presented on Figure 1. Most of the tested MABs recognize both monomer and dimer forms of HPV type 11.

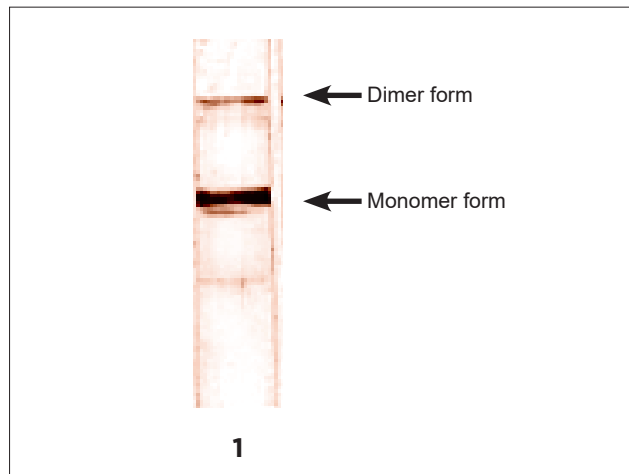


Figure 1. Detection of E7 HPV type 11 (conjugated with hsp70) in Western blotting by different monoclonal antibodies after 12% SDS-PAAG electrophoresis.

Strip 1: MAb 711-13
E7 HPV type 11 quantity: 15.0 µg/strip.

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 (see Figure 2)
716-D1 – 716-F10

MAbs 716-D1, 716-281, 716-325 are equally suitable for capture of both HPV type 16 and 18.

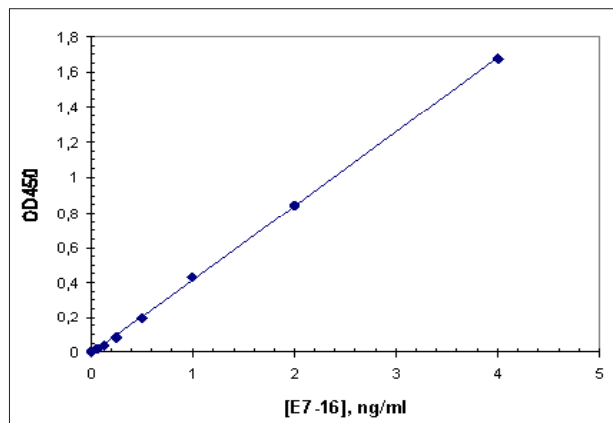


Figure 2. Calibration curves for E7 HPV type 16 sandwich immunoassays: 716-D1 - 716-332.

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 immunodetection in Western blotting after antigen SDS-gel electrophoresis and its transfer onto nitrocellulose membrane are presented on Figure 3. Most of the tested MABs recognize both monomer and dimer (most common in physiological media) forms of HPV type 16.

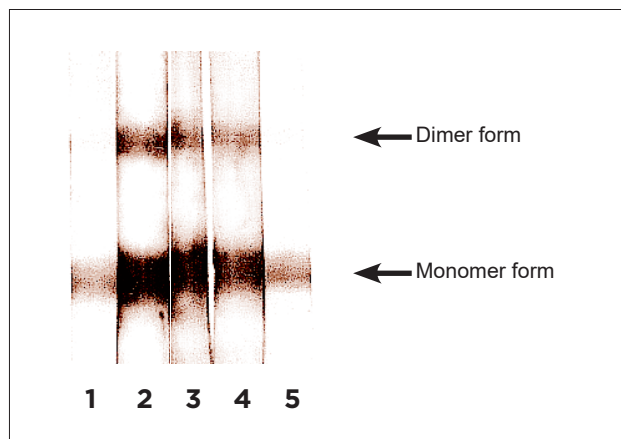


Figure 3. Detection of E7 HPV type 16 in Western blotting by different monoclonal antibodies after 15% SDS-PAAG electrophoresis.

Strip 1: MAb 716-281
Strip 2: MAb 716-332
Strip 3: MAb 716-D1
Strip 4: MAb 716-E11
Strip 5: MAb 716-F10
E7 HPV type 16 quantity: 15.0 µg/strip.

Monoclonal antibodies specific to HPV, type 18, oncoprotein E7

E7 HPV type 18 immunodetection in ELISA

The best combination of monoclonal antibodies for E7 HPV type 18 sandwich ELISA were selected from several MAb combinations. The pairs were selected on the basis of both MAb mapping data (maximal spatial determinant separation), sensitivity, specificity and kinetics characteristics.

Recommended pairs for sandwich ELISA are (capture - detection):

716-D1 - 718-238 (see Figure 4)
718-67 - 718-238

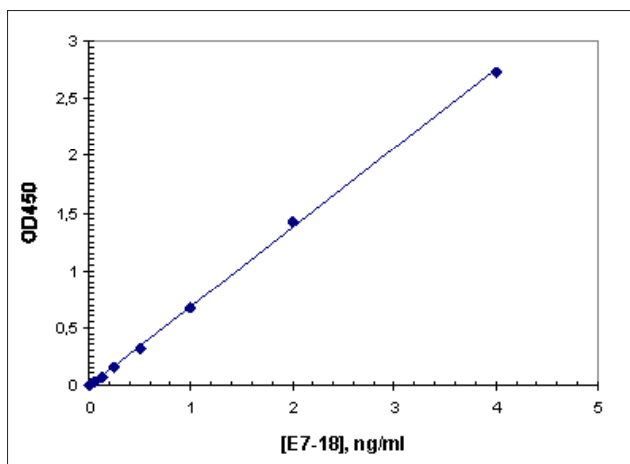


Figure 4. Calibration curves for E7 HPV type 18 sandwich immunoassays: 716-D1 - 718-238

Coating: MAb 716-D1 5 µg/ml, 0.1 M Carbonate buffer, pH 9.2
Detection: HRP-conjugated MAb 716-238, 1/20 000
Substrate: TMB

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 5. As can be seen, two tested MAbs, 716-281 and 716-D1, have 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. MAbs 718-67, 718-85 and 718-238 were found to be able to recognize both monomer and dimer forms of HPV type 18 and can be recommended for an ELISA application.

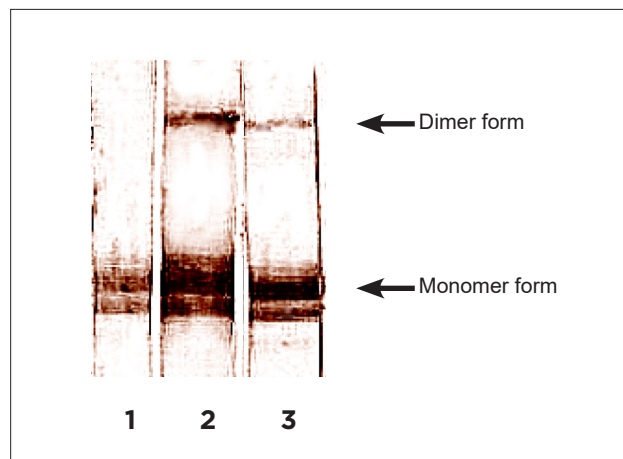


Figure 5. Detection of E7 HPV type 18 in Western blotting by different monoclonal antibodies after 15% SDS-PAAG electrophoresis:

Strip 1: MAb 718-15
Strip 2: MAb 718-67
Strip 3: MAb 718-238
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 11, 16 and 18 was done in indirect ELISA (coating 5 mg/ml of each antigen; MAbs 3 mg/ml.)

MAb	Isotype	Immunogen (hsp70 conjugated):		Cross reactivity with:		
		HPV type	E7 oncoprotein fragment	E7, type 11	E7, type 16	E7, type 18
711-13	IgG1	11	Whole molecule	100%	100%	89%
716-281	IgG2b	16	Whole molecule	0%	100%	37%
716-325	IgG2a	16	Whole molecule	0%	100%	0%
716-332	IgG1	16	Whole molecule	0%	100%	43%
716-D1	IgG2a	16	Whole molecule	0%	100%	114%
716-F10	IgG1	16	Whole molecule	0%	100%	100%
718-15	IgG1	18	Whole molecule	7%	43%	100%
718-67	IgG2a	18	Whole molecule	0%	9%	100%
718-238	IgG2b	18	Whole molecule	0%	8%	100%

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Human papillomavirus, type 11, oncoprotein E7	3HP11	711-13	IgG1	EIA, WB, C/r with types 16, 18
Human papillomavirus, type 16, oncoprotein E7	3HP16	716-281	IgG2b	EIA, WB, C/r with type 18
		716-325	IgG2a	EIA, WB
		716-F10	IgG1	EIA, WB, C/r with type 18
		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
		718-238	IgG2b	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