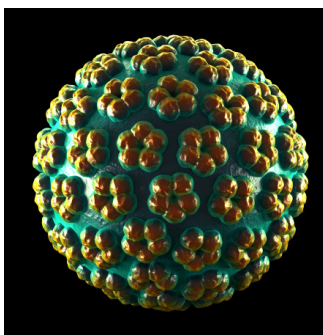


# Human Papillomavirus (HPV)



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, 3 etc.). Today genital HPV infection is one of the most wide-spread 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.

**Advanced ImmunoChemical 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 6 and 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.

# 1. HPV, type 6, oncoprotein E7 monoclonal antibodies

Host animal: Mice Balb/c  
Cell line used for fusion: Sp2/0  
Immunogen: Recombinant oncoprotein E7, type 6, conjugated with hsp70  
Specificity: Human papilloma virus, type 6 (for cross reactivity information see Fig. 1)  
Purification method: Protein G affinity chromatography

## 1.1. Applications

MAb 706-C5 against E7 HPV type 6 can be used in routine immunoassays like HPV enzyme immunoassay and HPV immunodetection in Western blotting. It should be noted that it is cross-reacting with HPV types 11, 16 and 18 (Fig. 1).

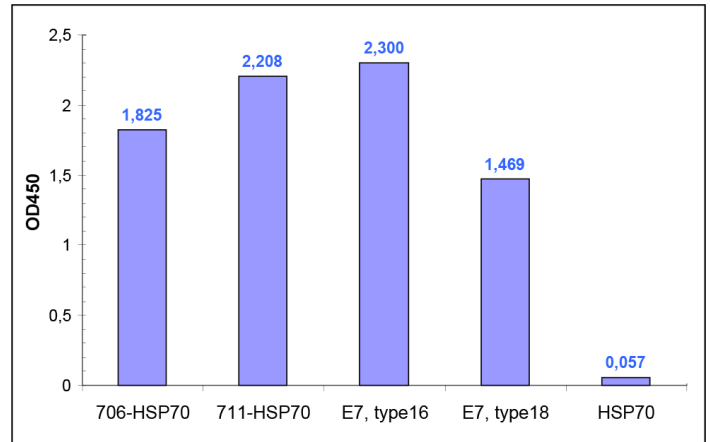


Figure 1. Testing of MAb 706-C5 cross-reactivity in indirect ELISA. Coating: 1 mg/ml of each antigen; MAb 706-C5, 3 mg/ml

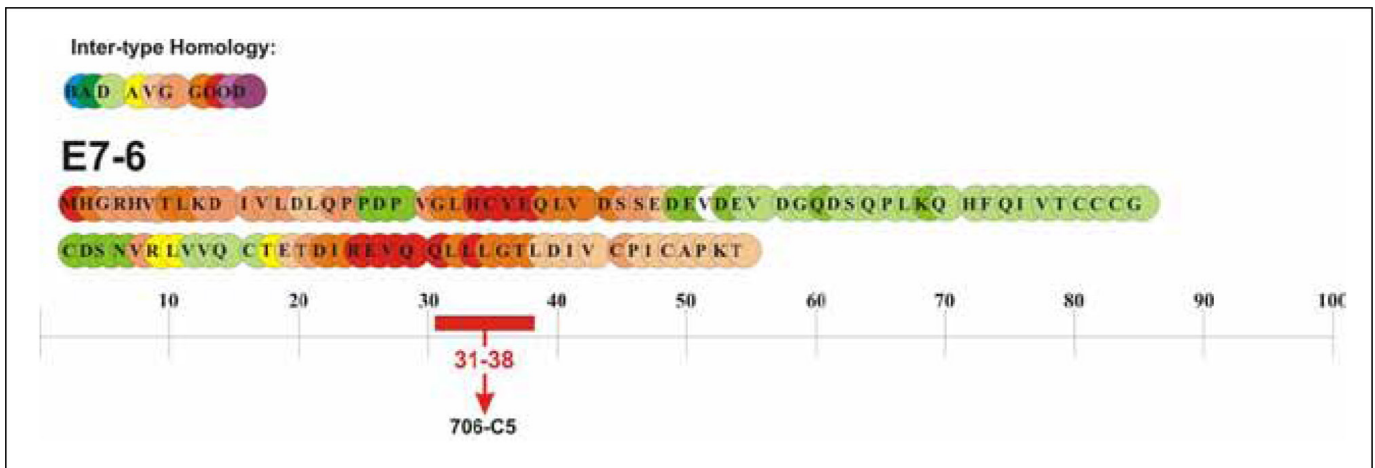


Figure 2. Results of the MAb mapping.

## 2. HPV, type 11, oncoprotein E7 monoclonal antibodies

Host animal: Mice Balb/c  
 Cell line used for fusion: Sp2/0  
 Immunogen: Recombinant oncoprotein E7, type 11, conjugated with hsp70  
 Specificity: Human papilloma virus, type 11 (for cross reactivity information see table 2)  
 Purification method: Protein G affinity chromatography

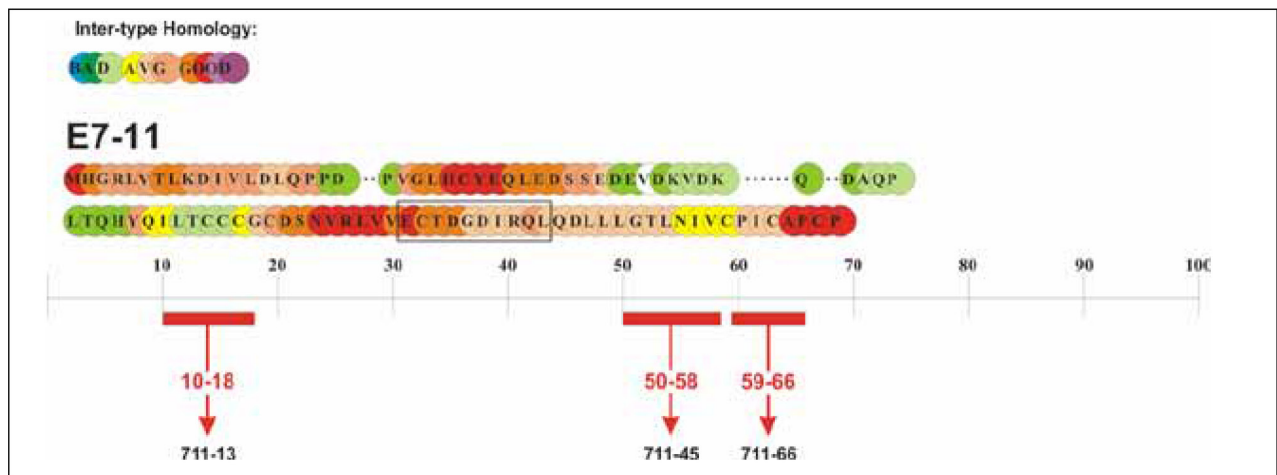
**Table 1. MAbs main characteristics.**

MAb	MAb isotype	Immunogen (hsp70 conjugated)	
		HPV type	E7 oncoprotein fragment
711-13	IgG1	11	Whole molecule
711-45	IgG2a	11	Whole molecule
711-66	IgG1	11	Whole molecule

**Table 2. MAbs specificity.**

Study of cross-reactivity with HPV types 11, 16 and 18 Indirect ELISA, Coating: 5 mg/ml of each antigen; MAbs: 3 mg/ml

MAbs	Cross reactivity with		
	E7, type 11	E7, type 16	E7, type 18
711-13	100%	100%	89%
711-45	100%	38%	27%
711-66	100%	12%	12%



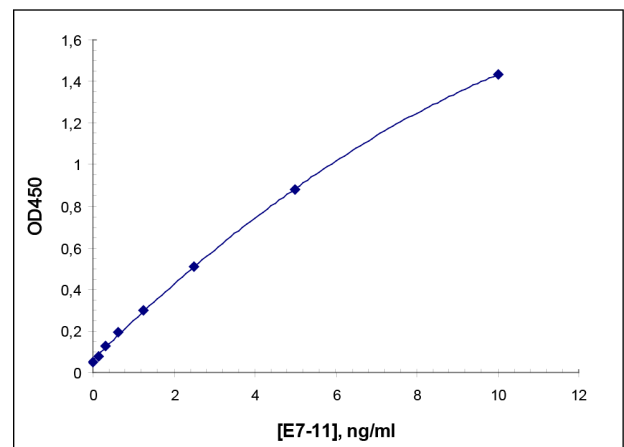
**Figure 3. Results of the MAbs mapping.**

### 2.1. E7 HPV type 11 immunodetection in ELISA

The best combination of monoclonal antibodies for E7 HPV type 11 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):

- 711-45 – 711-13 (see Fig. 4)
- 711-66 – 711-13



**Figure 4. Calibration curves for E7 HPV type 11 sandwich immunoassays: 711-45 – 711-13.**

Coating: MAb 711-45, 5 µg/ml in 0.1 M, Carbonate buffer, pH 9.2

Detection: HRP-conjugated MAb 711-13, 1/20 000

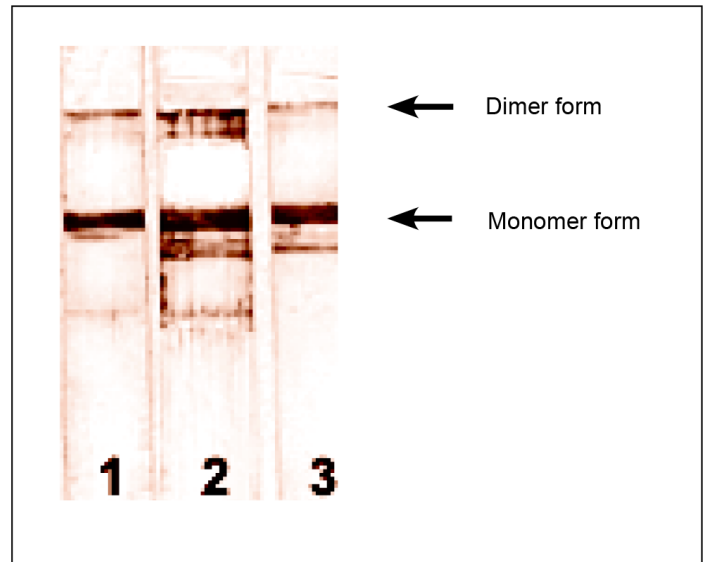
Substrate: TMB

## 2.2. 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 Fig. 5. Most of the tested MAbs recognize both monomer and dimer forms of HPV type 11.

**Figure 5. 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, Strip 2: MAb 711-45, Strip 3: MAb 711-66, E7 HPV type 11 quantity: 15.0 µg/strip.



## 3. HPV, type 16, oncoprotein E7 monoclonal antibodies

Host animal: Mice Balb/c  
 Cell line used for fusion: Sp2/0  
 Antigen: Recombinant oncoprotein E7, type 16 (whole or fragments), conjugated with hsp70  
 Specificity: Human papilloma virus, type 16 (for cross reactivity information see table 4)  
 Purification method: Protein G affinity chromatography

**Table 3. MAbs main characteristics.**

MAb	MAb isotype	Immunogen (hsp70 conjugated)	
		HPV type	E7 oncoprotein fragment
716-281	IgG2b	16	Whole molecule
716-325	IgG2a	16	Whole molecule
716-332	IgG1	16	Whole molecule
716-A6**	IgG2a	16	Whole molecule
716-B2**	IgG1	16	Whole molecule
716-C4**	IgG1	16	Whole molecule
716-D1	IgG2a	16	Whole molecule
716-E11	IgG1	16	Whole molecule
716-F10	IgG1	16	Whole molecule
ST1-A8**	IgG1	16	36-54 a.a.r. fragment
ST1-A9**	IgG1	16	36-54 a.a.r. fragment
ST1-B7**	IgG1	16	36-54 a.a.r. fragment
ST1-B11**	IgG1	16	36-54 a.a.r. fragment
Com1-D9**	IgG1	16	CR1 fragment (common for all E7)
Com2-D9**	IgG1	16	CR2 fragment (common for all E7)
Com2-C11**	IgG1	16	CR2 fragment (common for all E7)
Com2-D4**	IgG1	16	CR2 fragment (common for all E7)

\*\* MAb available only on special request.

**Table 4. MAbs specificity.**

Study of cross-reactivity with HPV types 11, 16 and 18 Indirect ELISA, Coating: 5 mg/ml of each antigen; MAbs: 3 mg/ml

MAbs	Cross reactivity with		
	E7, type 11	E7, type 16	E7, type 18
716-281	0%	100%	37%
716-325	0%	100%	0%
716-332	0%	100%	43%
716-A6**	0%	100%	9%
716-B2**	0%	100%	83%
716-C4**	0%	100%	42%
716-D1	0%	100%	114%
716-E11	0%	100%	97%
716-F10	0%	100%	100%
Com1-D9** *)	0%	13%	0%
Com2-D9** *)	0%	35%	100%
Com2-C11** *)	0%	0%	21%

\*\* MAb available only on special request.

\*) 100% reaction with corresponding peptide CR1 and CR2

## 4 AssayNotes | Human Papillomavirus (HPV)

### 3.1. 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.

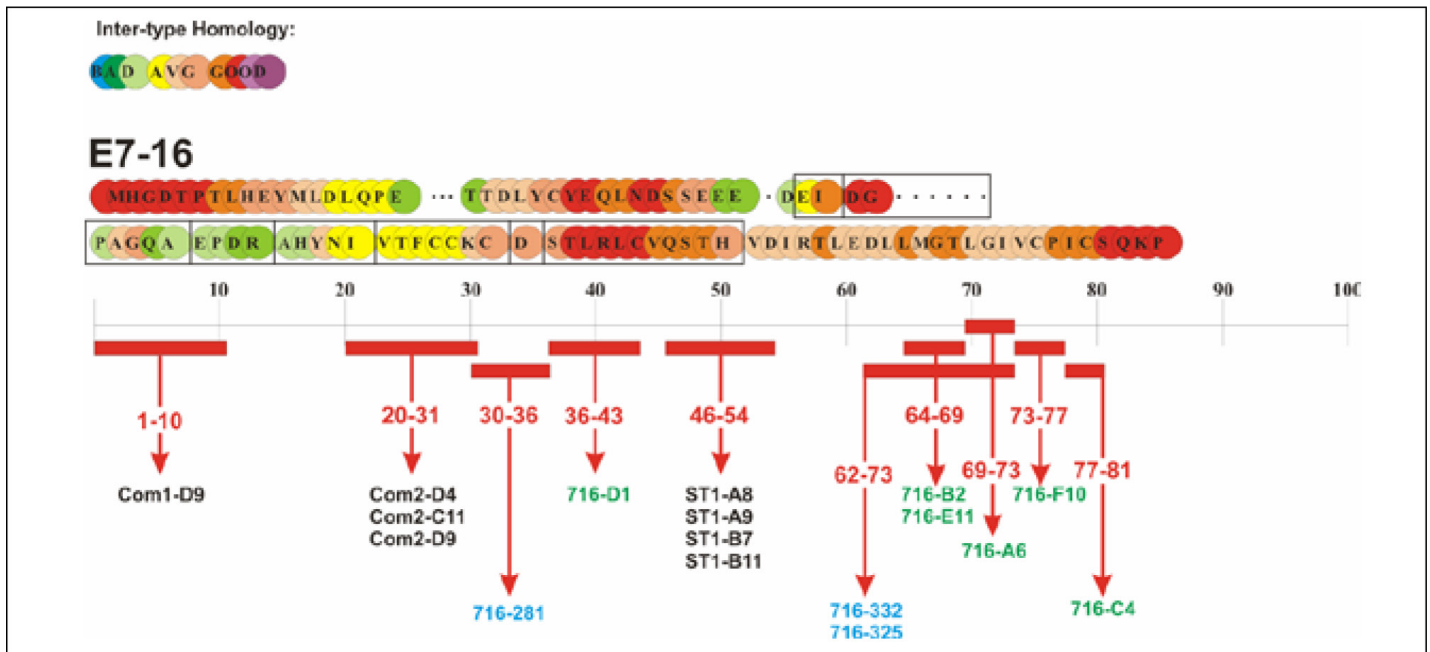


Figure 6. Results of the MAbs mapping.

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

- 716-D1 – 716-332 (see Fig. 7)
- 716-D1 – 716-E11
- 716-D1 – 716-F10

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

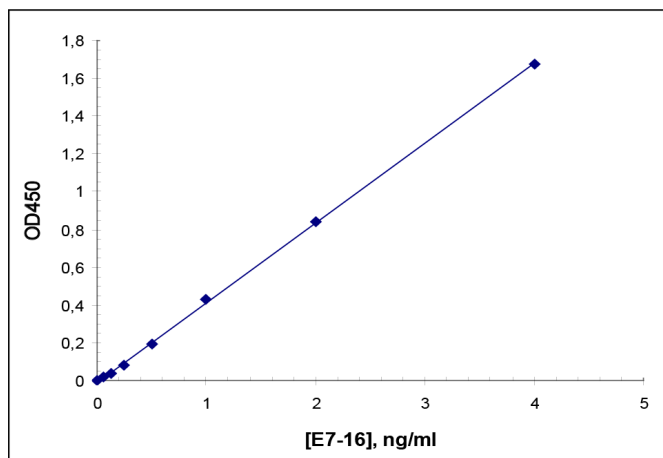


Figure 7. Calibration curves for E7 HPV type 16 sandwich immunoassays: 716-D1 - 716-332.

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

### 3.2. 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 Fig. 8. Most of tested MAbs recognize both monomer and dimer (most common in physiological media) forms of HPV type 16.

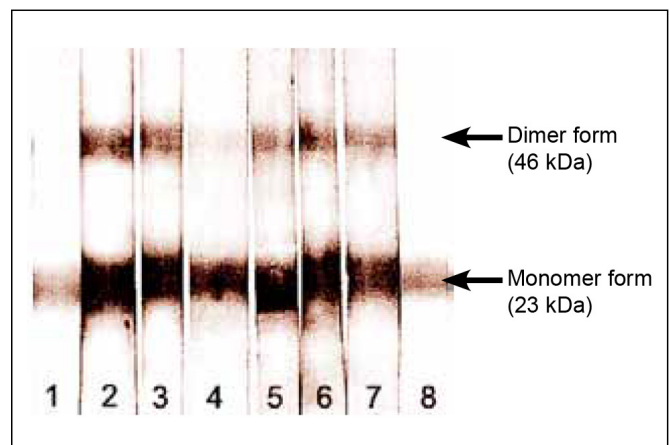


Figure 8. Detection of E7 HPV type 16 in Western blotting by different monoclonal antibodies after 15% SDS-PAAG electrophoresis. Strip 1: MAb 716-281, Strip 2: MAb 716-332, Strip 3: MAb 716-A6, Strip 4: MAb 716-B2, Strip 5: MAb 716-C4, Strip 6: MAb 716-D1, Strip 7: MAb 716-E11, Strip 8: MAb 716-F10; E7 HPV type 16 quantity: 15.0 µg/strip.

## 4. HPV, type 18, oncoprotein E7 monoclonal antibodies

Host animal: Mice Balb/c  
 Cell line used for fusion: Sp2/0  
 Antigen: Recombinant oncoprotein E7, types 18 (whole or fragments), conjugated with hsp70  
 Specificity: Human papilloma virus, type 18 (for cross reactivity information see table 5)  
 Purification method: Protein G affinity chromatography

Table 5. MAbs main characteristics.

MAb	MAb isotype	Immunogen (hsp70 conjugated)	
		HPV type	E7 oncoprotein fragment
718-15	IgG1	18	Whole molecule
718-67	IgG2a	18	Whole molecule
718-85**	IgG2b	18	Whole molecule
718-238	IgG2b	18	Whole molecule
718-A7**	IgG2a	18	Whole molecule
718-B5**	IgG1	18	Whole molecule
718-B6**	IgG1	18	Whole molecule
718-F9**	IgG2b	18	Whole molecule
718-G9**	IgG2a	18	Whole molecule

Table 6. MAbs specificity.

Study of cross-reactivity with HPV types 11, 16 and 18 Indirect ELISA, Coating: 5 mg/ml of each antigen; MAbs: 3 mg/ml

MAb	Cross reactivity with		
	E7, type 11	E7, type 16	E7, type 18
718-15	7%	43%	100%
718-67	0%	9%	100%
718-85**	0%	32%	100%
718-238	0%	8%	100%
718-B6**	5%	43%	100%
718-F9**	0%	21%	100%
718-G9**	0%	13%	100%

\*\* MAb available only on special request

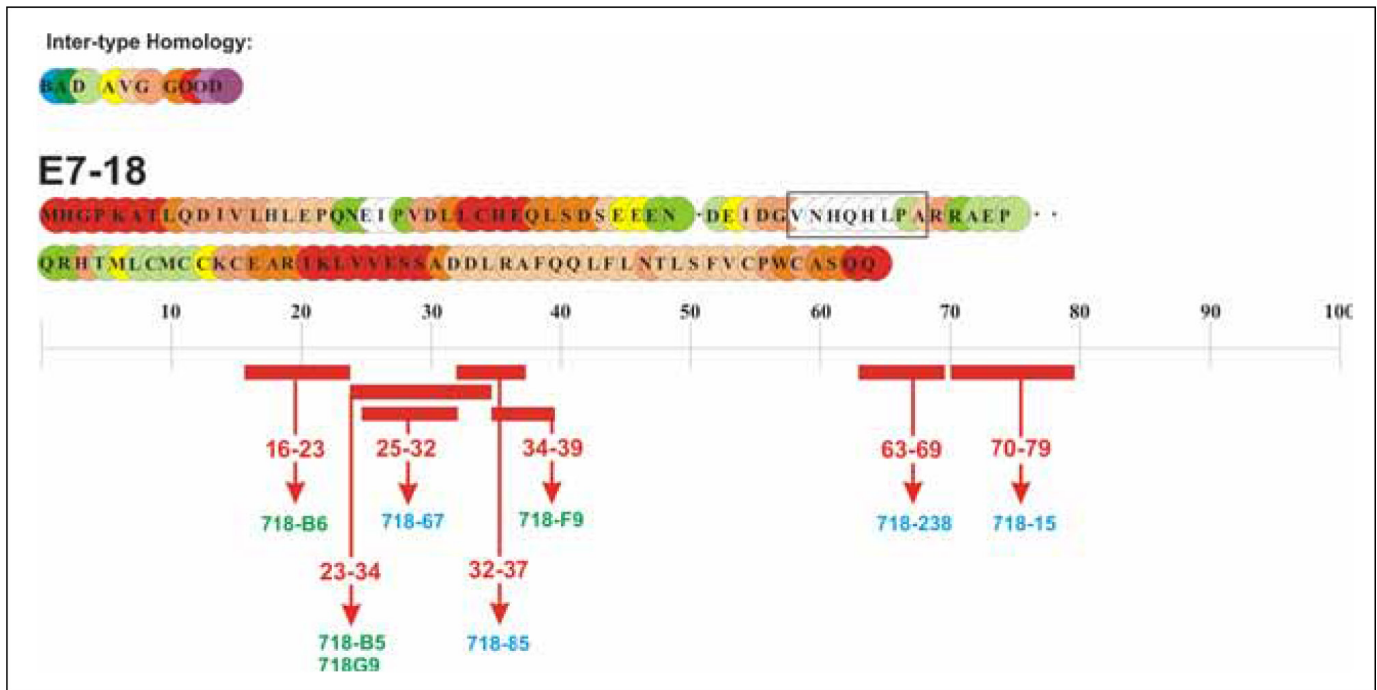


Figure 9. Results of the MAbs mapping.

#### 4.1. 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 Fig. 10)
- 718-15 – 718-85
- 718-67 – 718-238

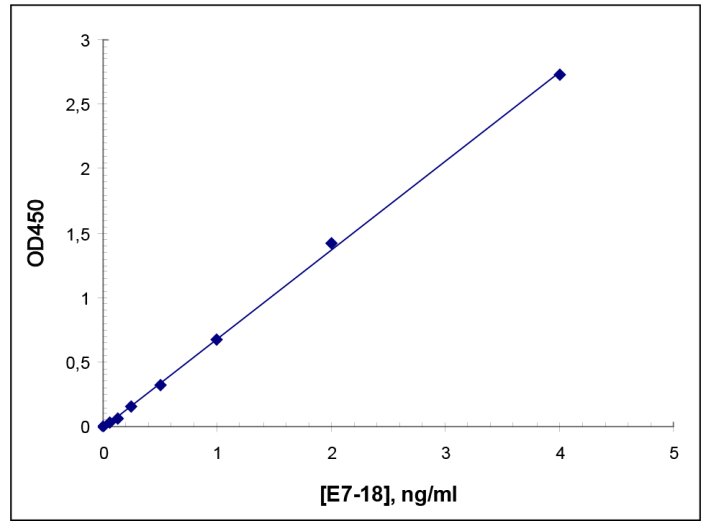


Figure 10. Calibration curves for E7 HPV type 18 sandwich immunoassays: 716-D1 – 718-238

Coating: MAb 716-D1 5  $\mu$ g/ml, 0.1 M Carbonate buffer, pH 9.2

Detection: HRP-conjugated MAb 716-238, 1/20 000

Substrate: TMB

#### 4.2. 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 on Fig. 11. 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.

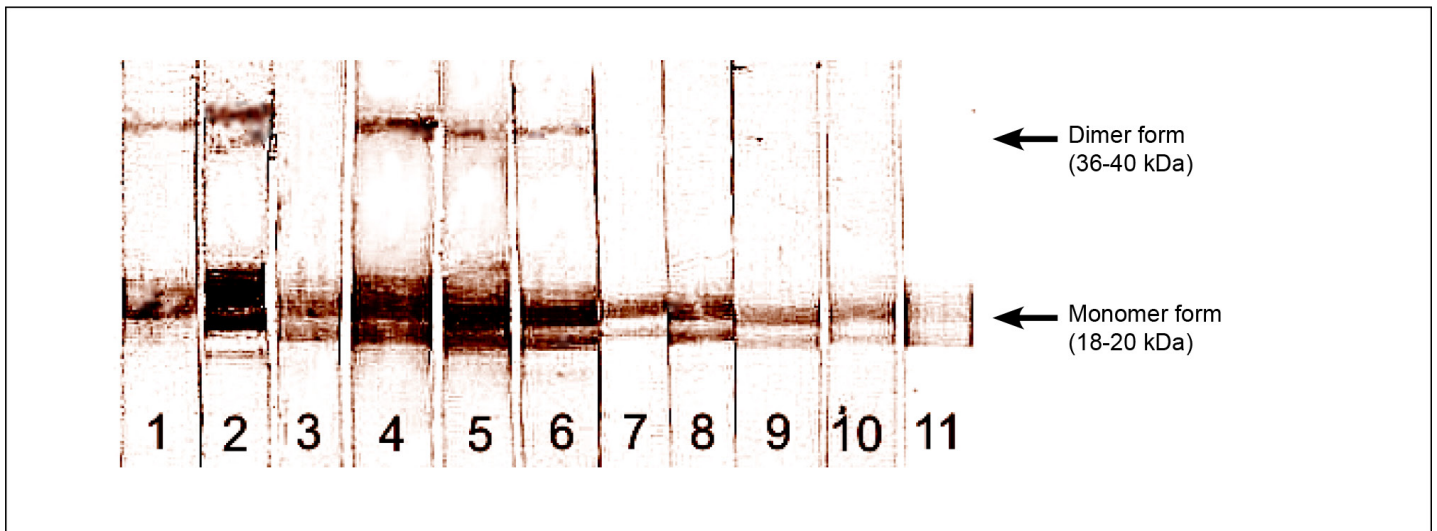


Figure 11. Detection of E7 HPV type 18 in Western blotting by different monoclonal antibodies after 15% SDS-PAAG electrophoresis:

Strip 1: MAb 716-281, Strip 2: MAb 716-D1, Strip 3: MAb 718-15, Strip 4: MAb 718-67, Strip 5: MAb 718-85, Strip 6: MAb 718-238, Strip 7: MAb 718-A7; Strip 8: MAb 718-B5; Strip 9: MAb 718-B6; Strip 10: MAb 718-F9; Strip 11: MAb 718-G9; E7. HPV type 18 quantity: 15.0  $\mu$ g/strip.

## Ordering Information:

MAb	Cat #	MAb	Subclass	Remarks
Anti-E7 HPV type 11	5-HPV11	711-13	IgG1	EIA, WB, C/r with types 11, 16 and 18
Anti-E7 HPV type 11	5-HPV11	711-45	IgG2a	EIA, WB
Anti-E7 HPV type 11	5-HPV11	711-66	IgG3	EIA, WB
Anti-E7 HPV type 16	5-HPV16E7	716-281	IgG2b	EIA, WB
Anti-E7 HPV type 16	5-HPV16E7	716-325	IgG2a	EIA (capture), WB
Anti-E7 HPV type 16	5-HPV16E7	716-332	IgG1	EIA (detection), WB
Anti-E7 HPV type 16	5-HPV16E7	716-A6**	IgG2a	EIA (detection), WB, dimer and monomer
Anti-E7 HPV type 16	5-HPV16E7	716-B2**	IgG1	WB, dimer and monomer
Anti-E7 HPV type 16	5-HPV16E7	716-C4**	IgG1	WB
Anti-E7 HPV type 16	5-HPV16E7	716-D1	IgG2a	WB, dimer and monomer
Anti-E7 HPV type 16	5-HPV16E7	716-F10	IgG1	EIA (capture), WB, dimer and monomer
Anti-E7 HPV type 16	5-HPV16E7	716-E11	IgG1	EIA (detection), WB, dimer and monomer
Anti-E7 HPV type 16	5-HPV16E7	ST1-A8**	IgG1	EIA (detection), WB
Anti-E7 HPV type 16	5-HPV16E7	ST1-A9**	IgG1	WB
Anti-E7 HPV type 16	5-HPV16E7	ST1-B7**	IgG1	WB
Anti-E7 HPV type 16	5-HPV16E7	ST1-B11**	IgG1	WB
Anti-E7 HPV type 16	5-HPV16E7	Com1-D9**	IgG1	WB
Anti-E7 HPV type 16	5-HPV16E7	Com2-D9**	IgG1	WB
Anti-E7 HPV type 16	5-HPV16E7	Com2-C11**	IgG1	WB
Anti-E7 HPV type 16	5-HPV16E7	Com2-D4**	IgG1	WB
Anti-E7 HPV type 18	5-HPV18E7	718-15	IgG1	EIA (capture), WB
Anti-E7 HPV type 18	5-HPV18E7	718-67	IgG2a	EIA (capture), WB
Anti-E7 HPV type 18	5-HPV18E7	718-85**	IgG2b	EIA (detection), WB
Anti-E7 HPV type 18	5-HPV18E7	718-238	IgG2b	EIA (detection), WB
Anti-E7 HPV type 18	5-HPV18E7	718-A7**	IgG2a	WB
Anti-E7 HPV type 18	5-HPV18E7	718-B5**	IgG1	WB
Anti-E7 HPV type 18	5-HPV18E7	718-B6**	IgG1	WB
Anti-E7 HPV type 18	5-HPV18E7	718-F9**	IgG2b	WB
Anti-E7 HPV type 18	5-HPV18E7	718-G9**	IgG2a	WB

\*\*MAb available only upon special request

## 5. Human papilloma virus (HPV) antigens

Product Name	Cat #	Purity	Source
Human Papillomavirus L1 protein (HPVL1), type 16	8-HPVL16	>90%	Recombinant
Human Papillomavirus L1 protein (HPVL1), type 18	8-HPVL18	>90%	Recombinant

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