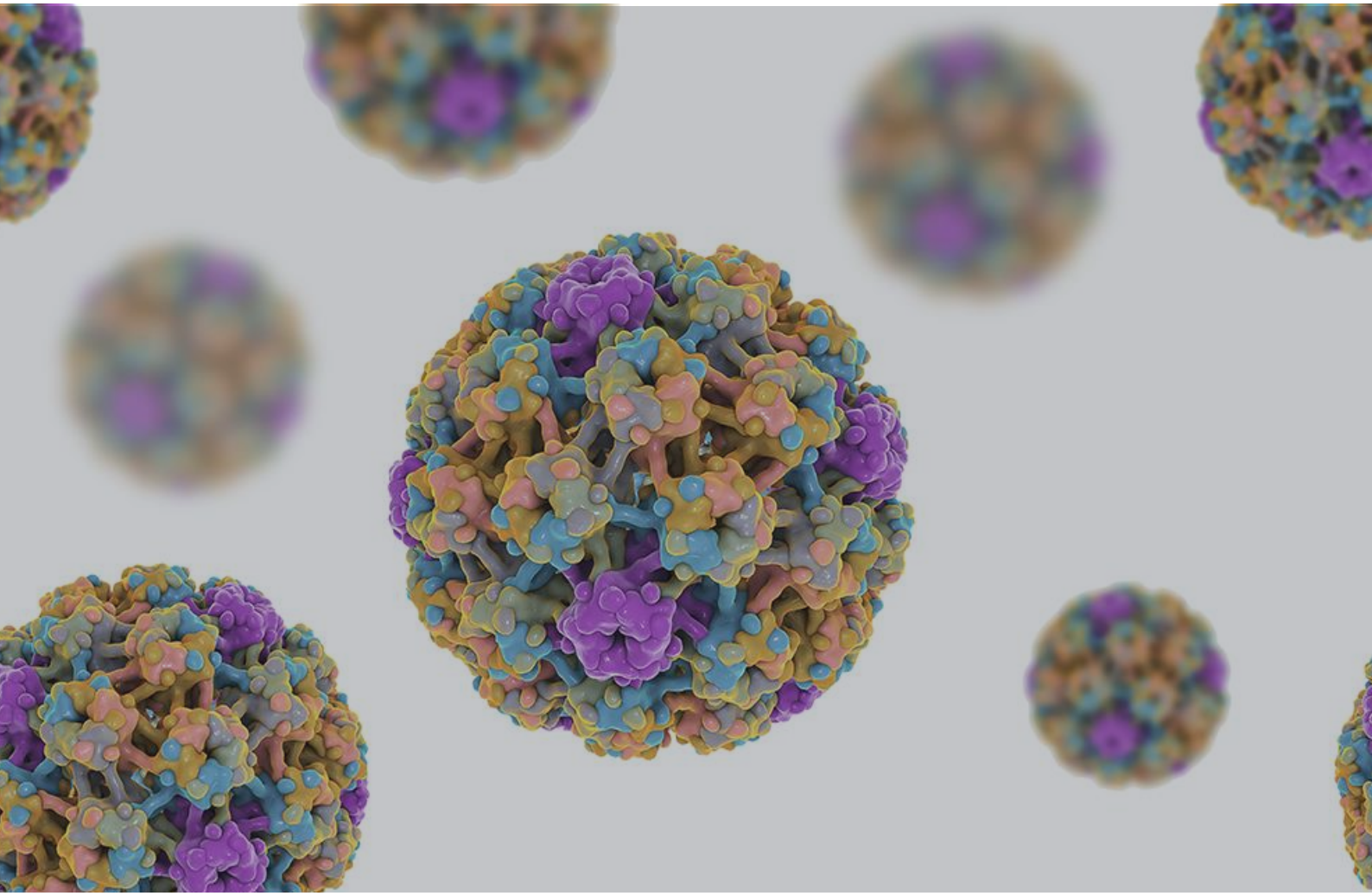
HPV Reagents for Vaccine Research and Immunoassay Development



Key HPV L1 Antibodies, VLPs, ELISA Kits and Pseudovirus for Vaccine Research

CD offers a wide spectrum of monoclonal antibodies specific to oncoprotein E7 of "high-risk" HPV types 16 and 18 for immunoassay development.

CD Creative Diagnostics®

Content

Introduction....3
HPV L1 Monoclonal Antibodies for Neutralization Assay....4
HPV L1 Polyclonal Antibodies For WB and ELISA....5
Recombinant HPV L1 VLPs....6
Pseudotyped GFP HPV....7
HPV E6/E7 Antibodies & Antigens....9
HPV ELISA Kits....10
CONTACT....10

Introduction

Human papillomavirus (HPV) is a virus from the papillomavirus family that is capable of infecting humans. Like all papillomaviruses, HPVs establish productive infections only in keratinocytes of the skin or mucous membranes. There are nearly 200 different strains of HPV, most of which are harmless and not cancer causing. Out of all these 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are known to lead to cancers of the cervix, vulva, vagina, penis, oropharynx and anus, and another 6 are suspected of causing cancer. In addition, HPV has been linked with an increased risk of cardiovascular disease.

They are made of a small, naked, icosahedral capsid (55 nm in diameter) that encloses a circular, double-stranded DNA genome whose organization is common to all papillomaviruses. The genome includes a non-coding region (upstream regulatory region), a region that codes for non-structural genes, mostly expressed early in the viral cycle (E1, E2, E4, E5, E6, and E7), and a region that encodes the only two capsid proteins, which are expressed late (L1 and L2). The sequence of L1 gene is what defines the distinct HPV genotypes.

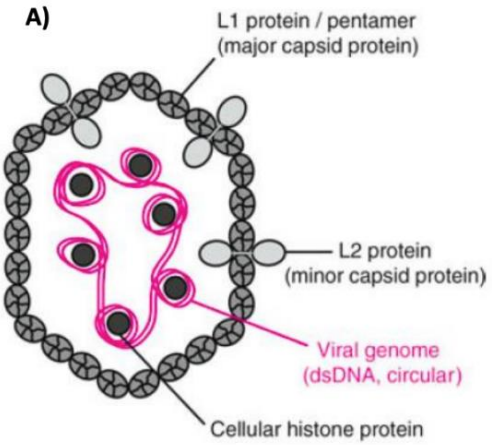


Figure 1: HPV structure

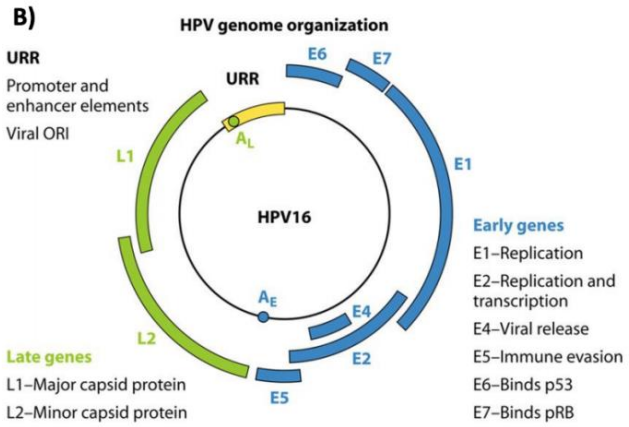


Figure 2: HPV genome organization

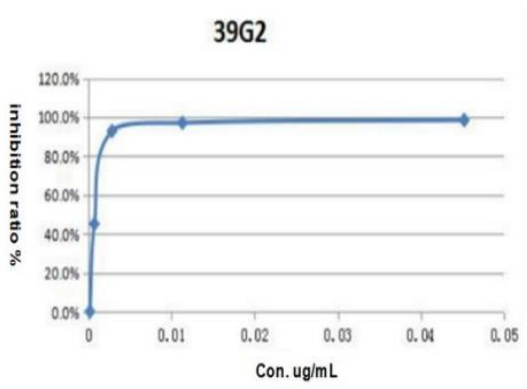
- E1: Encodes a protein that binds to the viral origin of replication in the long control region of the viral genome. E1 uses ATP to exert a helicase activity that forces apart the DNA strands, thus preparing the viral genome for replication by cellular DNA replication factors.
- E2: E2 facilitates the binding of E1 to the viral origin of replication. E2 also utilizes a cellular protein known as Bromodomain-4 (Brd4) to tether the viral genome to cellular chromosomes.
- E3: This small putative gene exists only in a few papillomavirus types. The gene is not known to be expressed as a protein and does not appear to serve any function.
- E4: The E4 protein of many papillomavirus types is thought to facilitate virion release into the environment by disrupting intermediate filaments of the keratinocyte cytoskeleton. Viral mutants incapable of expressing E4 do not support high-level replication of the viral DNA, but it is not yet clear how E4 facilitates DNA replication. E4 has also been shown to participate in arresting cells in the G2 phase of the cell cycle.
- E5: The E5 are small, very hydrophobic proteins that destabilize the function of many membrane proteins in the infected cell. The E5 proteins seem to activate the signal cascade initiated by epidermal growth factor upon ligand binding. HPV16 E5 and HPV2 E5 have also been shown to down-regulate the surface expression of major histocompatibility complex class I proteins, which may prevent the infected cell from being eliminated by killer T cells.
- E6: Its major role is to mediate the degradation of p53, a major tumor suppressor protein, reducing the cell's ability to respond to DNA damage.E6 has also been shown to target other cellular proteins, thereby altering several metabolic pathways. It is an appealing target of therapeutic HPV vaccines designed to eradicate established cervical cancer tumors.
- E7: The primary function of the E7 protein is to inactivate members of the pRb family of tumor suppressor proteins. Together with E6, E7 serves to prevent cell death (apoptosis) and promote cell cycle progression, thus priming the cell for replication of the viral DNA. E7 also participates in immortalization of infected cells by activating cellular telomerase. As with E6, the ongoing expression of E7 is required for survival of cancer cell lines, such as HeLa, that are derived from HPV-induced tumors.

- L1: L1 spontaneously self-assembles into pentameric capsomers. Purified capsomers can go on to form capsids, which are stabilized by disulfide bonds between neighboring L1 molecules. L1 capsids assembled in vitro are the basis of prophylactic vaccines against several HPV types. Compared to other papillomavirus genes, the amino acid sequences of most portions of L1 are well-conserved between types.
- L2: L2 exists in an oxidized state within the papillomavirus virion, with the two conserved cysteine residues forming an intramolecular disulfide bond. In addition to cooperating with L1 to package the viral DNA into the virion, L2 has been shown to interact with a number of cellular proteins during the infectious entry process.

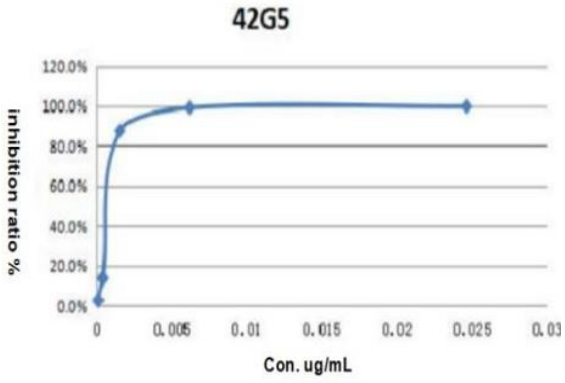
HPV infections are largely shielded from the host immune response because they are nonlytic and restricted to the epithelium. Serum antibodies against many different viral products have been demonstrated. The best characterized antibodies are those directed against conformational epitopes of the L1 capsid protein assembled as VLPs. Like most non-enveloped viruses, the capsid is geometrically regular and presents icosahedral. Self-assembled virus-like particles composed of L1 are the basis of a successful group of prophylactic HPV vaccines designed to elicit virus-neutralizing antibodies that protect against initial HPV infection. Compared to other papillomavirus genes, the amino acid sequences of most portions of L1 are well-conserved between types. However, the surface loops of L1 can differ substantially, even for different members of a particular papillomavirus species. This probably reflects a mechanism for evasion of neutralizing antibody responses elicited by previous papillomavirus infections.

HPV L1 Monoclonal Antibodies for Neutralization Assay

Figure 1. IC50 of Mab clones for HPV6 in pseudovirus-based neutralization assay:



IC50 (μg/ml) of 39G2 for Neut is 0.00081



IC50 (μg/ml) of 42G5 for Neut is 0.00076

Table 1. MAbs main characteristics: ELISA Results (O.D) evaluate the cross-reactivity between HPV6 and other types:

clone	HPV6	HPV11	HPV31	HPV33	HPV45	HPV52	HPV16	HPV18	HPV58
1E5	2.255	0.058	0.05	0.089	0.151	0.041	0.046	0.051	0.044
39G7	2.835	0.076	0.058	0.069	0.065	0.232	0.066	0.069	0.059
39G2	2.712	0.068	0.061	0.065	0.049	0.08	0.059	0.052	0.054
42G5	2.834	0.163	0.046	0.048	0.043	0.076	0.048	0.049	0.045
44B11	2.576	0.043	0.04	0.045	0.039	0.097	0.044	0.041	0.041
43C7	2.689	1.796	0.054	0.075	0.042	0.311	0.074	0.474	0.15

PERFORMANCE

- These antibodies are obtained by immunization and screening of L1-VLP expressed in E. coli.
- They may have no response or weak response to L1 expressed in other different systems or different processes.
- Have been proved to be able to be used in ELISA, but can not be used in Western Blot.
- Neutralizing activity have been confirmed using Pseudotyped GFP HPV.
- Each clone can be sold separately.

Cat. No.	Product name	Clone number-IC50 (μg/ml) for Neut:
CABT-B8803	Anti-HPV 6 L1 monoclonal antibody (set)	1E5-0.0092; 39G7-0.0085; 39G2-0.00081; 42G5-0.00076; 44B11-0.00008;43C7-0.0033
CABT-B8804	Anti-HPV 11 L1 monoclonal antibody (set)	34C9-4.27; 35C1-15.87; 35H5-743; 34E5-43
CABT-B8805	Anti-HPV 16 L1 monoclonal antibody (set)	2A1-0.00033; 3D5-0.0021; 4G12-0.0020; 5A6-0.0024; 6C7-0.0020; 7B9-0.00027
CABT-B8806	Anti-HPV 18 L1 monoclonal antibody (set)	1B1-97; 3A2-0.00030; 3A4-1.46; 4H1-0.76; 4H5-1.31; 7H8-0.0025
CABT-B8807	Anti-HPV 31 L1 monoclonal antibody (set)	19C2-0.0061; 19B6-0.12
CABT-B8808	Anti-HPV 33 L1 monoclonal antibody (set)	
CABT-B8809	Anti-HPV 45 L1 monoclonal antibody (set)	30H6-0.54; 35B12-1.70; 35H9-0.49; 40H1-0.15; 46G5-0.028; 48B1-0.14
CABT-B8810	Anti-HPV 52 L1 monoclonal antibody (set)	36B9-0.00056; 36E12-0.00061; 40C2-0.0035; 41C3-0.0011; 41D1-0.00015; 42A2-0.00040
CABT-B8811	Anti-HPV 58 L1 monoclonal antibody (set)	1D2-0.015; 2E11-0.021; 2F11-0.014; 2F7-0.020; 2F9-0.10; 2G7-0.015
CABT-CS276	Anti-HPV 35 L1 monoclonal antibody (set)	17B7-17.11; 17G6-10.56
CABT-CS277	Anti-HPV 39 L1 monoclonal antibody (set)	13A2-0.069; 13B7-1.02
CABT-CS278	Anti-HPV 51 L1 monoclonal antibody (set)	4C1-0.012; 15C6-3.47
CABT-CS279	Anti-HPV 56 L1 monoclonal antibody (set)	5A3-16.60; 6F12-0.0043
CABT-CS280	Anti-HPV 59 L1 monoclonal antibody (set)	6D9-0.0024; 7D7-0.0012
CABT-CS281	Anti-HPV 68 L1 monoclonal antibody (set)	2C5-0.04; 8B6-0.00023

HPV L1 Polyclonal Antibodies For WB and ELISA

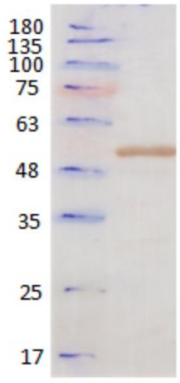


Figure 2. Detection of HPV type 51 L1 protein in Western blotting by CABT-CS040 after 10% SDS-PAGE

CABT-CS040 quantity: 1.0ug/strip(1ug/ml)

PERFORMANCE

- Each type can be supplied in unconjugated and HRP formats
- May have cross-reactivity to the L1 protein of other types of HPV
- Protein G purified
- Work well in ELISA and WB

Cat. No.	Polyclonal antibody	Cat. No.	HRP conjugated
CABT-B8785	Anti-HPV6 L1 polyclonal antibody	CABT-B8786	Anti-HPV6 L1 polyclonal antibody [HRP]
CABT-B8787	Anti-HPV11 L1 polyclonal antibody	CABT-B8788	Anti-HPV11 L1 polyclonal antibody [HRP]
CABT-B8789	Anti-HPV16 L1 polyclonal antibody	CABT-B8790	Anti-HPV16 L1 polyclonal antibody [HRP]
CABT-B8791	Anti-HPV18 L1 polyclonal antibody	CABT-B8792	Anti-HPV18 L1 polyclonal antibody [HRP]
CABT-B8793	Anti-HPV31 L1 polyclonal antibody	CABT-B8794	Anti-HPV31 L1 polyclonal antibody [HRP]
CABT-B8795	Anti-HPV33 L1 polyclonal antibody	CABT-B8796	Anti-HPV33 L1 polyclonal antibody [HRP]
CABT-B8797	Anti-HPV45 L1 polyclonal antibody	CABT-B8798	Anti-HPV45 L1 polyclonal antibody [HRP]
CABT-B8799	Anti-HPV52 L1 polyclonal antibody	CABT-B8800	Anti-HPV52 L1 polyclonal antibody [HRP]
CABT-B8801	Anti-HPV58 L1 polyclonal antibody	CABT-B8802	Anti-HPV58 L1 polyclonal antibody [HRP]
CABT-CS038	Anti-HPV 35-L1 Polyclonal antibody	CABT-CS282	Anti-HPV 35 -L1 Polyclonal antibody[HRP]
CABT-CS039	Anti-HPV 39-L1 Polyclonal antibody	CABT-CS283	Anti-HPV 39 -L1 Polyclonal antibody[HRP]
CABT-CS040	Anti-HPV 51-L1 Polyclonal antibody	CABT-CS284	Anti-HPV 51 -L1 Polyclonal antibody[HRP]
CABT-CS041	Anti-HPV 56-L1 Polyclonal antibody	CABT-CS285	Anti-HPV 56 -L1 Polyclonal antibody[HRP]
CABT-CS042	Anti-HPV 59-L1 Polyclonal antibody	CABT-CS286	Anti-HPV 59 -L1 Polyclonal antibody[HRP]
CABT-CS043	Anti-HPV 68-L1 Polyclonal antibody	CABT-CS281	Anti-HPV 68 -L1 Polyclonal antibody[HRP]

Recombinant HPV L1 VLPs

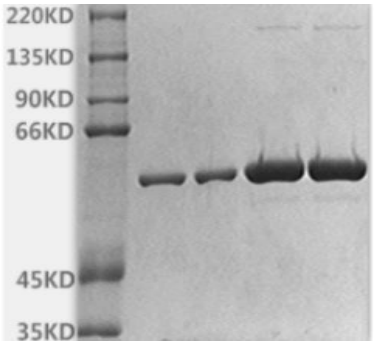


Figure 3. 10% SDS-PAGE result of purified HPV 6 VLP protein (DAGF-227)

Lane2.3: 2ug of DAGF-227
Lane4.5: 10ug of DAGF-227

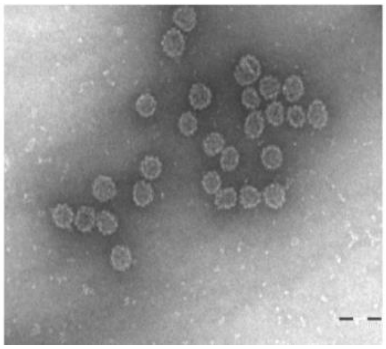


Figure 4. Analysis of HPV 6 VLP protein (DAGF-227) by electron microscopy.

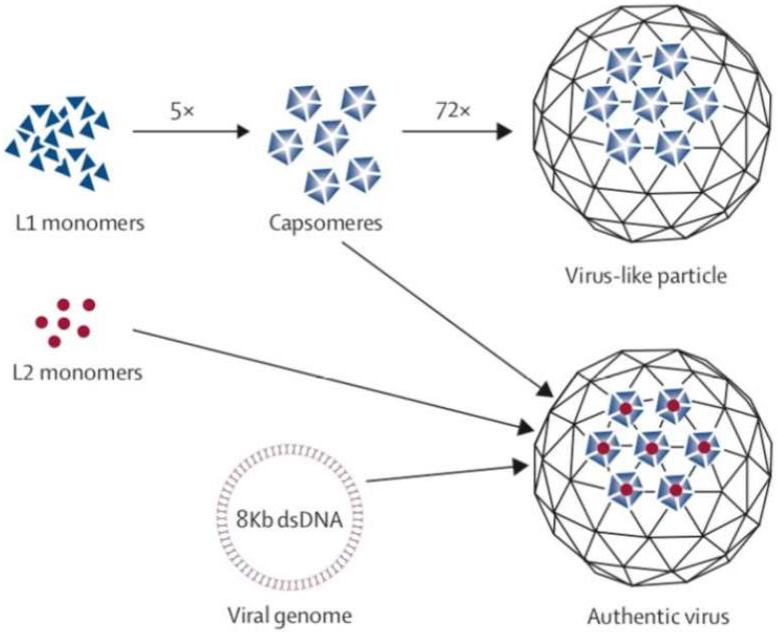
PERFORMANCE

- Purity> 95% (SDS-PAGE), manufactured in large scale
- May contain a small amount of dimer or trimer

Cat. No.	Product Description	Expression System	Application
DAGF-227	Recombinant HPV type 6 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGF-228	Recombinant HPV type 11 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGF-229	Recombinant HPV type 16 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGF-230	Recombinant HPV type 18 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGF-231	Recombinant HPV type 31 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGF-232	Recombinant HPV type 33 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGF-233	Recombinant HPV type 45 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGF-234	Recombinant HPV type 52 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGF-235	Recombinant HPV type 58 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGC142	Recombinant HPV type 35 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGC143	Recombinant HPV type 39 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGC144	Recombinant HPV type 51 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGC145	Recombinant HPV type 56 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGC146	Recombinant HPV type 59 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection
DAGC147	Recombinant HPV type 68 L1 protein (VLP)	E. Coli	ELISA, Antibody Detection

Pseudotyped GFP HPV

The CD Pseudotyped GFP HPV production is based on the transfection of a 293 cell line, 293FT, engineered to express high levels of SV40 large T antigen. The cells are co-transfected with codon-modified papillomavirus capsid genes, L1 and L2, together with a pseudogenome plasmid containing the SV40 origin of replication. Pseudovirus (PsV) encapsidating a GFP reporter plasmid can be used to develop a high-throughput in vitro neutralization assay in a 96-well plate format.



- Instead of using authentic viruses, pseudovirions with an encapsidated reporter can be used to avoid high risks and provide more direct measurements for antibodies.
- HPV Pseudovirus (PsV) is produced by transfecting 293FT with codon-modified papillomavirus capsid genes, L1 and L2, together with a pseudogenome plasmid containing the SV40 origin of replication.
- Pseudovirus (PsV) encapsulating a GFP reporter plasmid can be used to develop a high-throughput in vitro neutralization assay in a 96-well plate format.

Cat. No.	Product Name	Reporter
PSVG-HPV6	Pseudotyped GFP HPV6	GFP
PSVG-HPV11	Pseudotyped GFP HPV11	GFP
PSVG-HPV16	Pseudotyped GFP HPV16	GFP
PSVG-HPV18	Pseudotyped GFP HPV18	GFP
PSVG-HPV31	Pseudotyped GFP HPV31	GFP
PSVG-HPV33	Pseudotyped GFP HPV33	GFP
PSVG-HPV35	Pseudotyped GFP HPV35	GFP
PSVG-HPV39	Pseudotyped GFP HPV39	GFP
PSVG-HPV45	Pseudotyped GFP HPV45	GFP
PSVG-HPV51	Pseudotyped GFP HPV51	GFP
PSVG-HPV52	Pseudotyped GFP HPV52	GFP
PSVG-HPV56	Pseudotyped GFP HPV56	GFP
PSVG-HPV58	Pseudotyped GFP HPV58	GFP
PSVG-HPV59	Pseudotyped GFP HPV59	GFP
PSVG-HPV68	Pseudotyped GFP HPV68	GFP

HPV E6/E7 Antibodies & Antigens

CD 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).

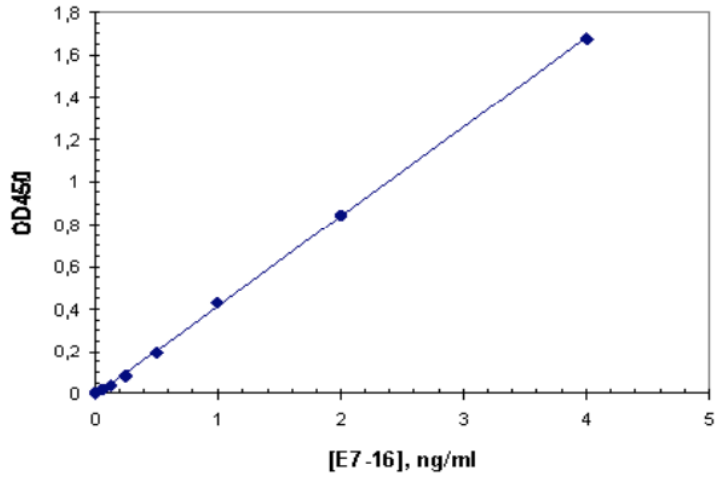


Figure 5. Calibration curves for E7 HPV type 16 sandwich ELISA: CABT-WN1070- CABT-WN1068.

Coating: CABT-WN1070, 5 mg/ml, 0.1 M Carbonate buffer, pH 9.2

Detection: HRP-conjugated MAb CABT-WN106, 1/50 000

Substrate: TMB

Target	Cat#	Product Name	Source	Application
HPV 16	DAGF-095	Recombinant HPV16 E6 protein (aa 1-158) [His]	Yeast	ELISA
	DAGF-094	Recombinant HPV16 E6 protein [His]	E.coli	ELISA
	DAGF-096	Recombinant HPV16 E7 protein (aa 1-98) [GST]	Yeast	ELISA
	CABT-WN1068	Anti-HPV16 E7 monoclonal antibody, clone 827-392	Mouse	ELISA(Det), WB
	CABT-WN1069	Anti-HPV16 E7 monoclonal antibody, clone 827-436	Mouse	ELISA(Det), WB
	CABT-WN1070	Anti-HPV16 E7 monoclonal antibody, clone C827N	Mouse	ELISA(Cap), WB
HPV 18	DAGF-097	Recombinant HPV18 E6 protein (aa 1-158) [His]	E. coli	ELISA
	DAGF-098	Recombinant HPV18 E7 protein (aa 1-105) [His]	Yeast	ELISA
	CABT-WN1074	Anti-HPV18 E7 monoclonal antibody, clone 829-26	Mouse	ELISA, WB
	CABT-WN1075	Anti-HPV18 E7 monoclonal antibody, clone 829-78	Mouse	ELISA, WB

HPV ELISA Kits

Cat. No.	Product Name
DEIASL118	Human HPV 16 Antigen ELISA Quantitation Kit
DEIASL119	Human HPV 18 Antigen ELISA Quantitation Kit
DEIASL120	Human HPV 52 Antigen ELISA Quantitation Kit
DEIASL121	Human HPV 58 Antigen ELISA Quantitation Kit
DEIASL171	Human HPV 16 E7 Oncoprotein ELISA Kit
DEIASL172	Human HPV 18 E7 Oncoprotein ELISA Kit
DEIASL404	Human HPV18 IgM ELISA kit
DEIASL405	Human HPV 16 L1-capsids IgG ELISA Kit
DEIASL406	Human HPV 16 IgM ELISA Kit
DEIASL408	Human HPV 18 L1-capsids IgG ELISA Kit
DEIASL410	Human high risk HPV L1-capsids (HR-HPVL1) IgG ELISA kit
DEIA-F678S	Human Papilloma Virus IgG ELISA Kit
DEIASL407	Human Papilloma Virus IgM ELISA Kit

CONTACT

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