STORAGE and STABILITY
The antibody should be stored at 2-8°C lyophilized or reconstituted. Expiration date on vial label applies to unreconstituted product. After reconstitution, product is stable for 2 months at 2°-8°C. For extended storage, aliquot and store at -20°C or below. Avoid repeated freezing and thawing.

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TRADEMARKS

Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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PI280006 (Rev 02/10) DCC-10-0498

invitrogen™

HPV16-E7
Mouse Monoclonal Antibody

Catalog No. 28-0006
Formerly Ciba-Corning Diagnostics' Product No. 100201

Contents: 1.0 mL (Lyophilized)

INTENDED USE
For research use only. CAUTION: Not intended for human or animal therapeutic or diagnostic use.

FORM
The product is supplied lyophilized. Prior to filling (1 mL/vial) and lyophilization, the reagent contains PBS, 1% BSA, and 0.1% sodium azide. This antibody is HPLC purified and is supplied at a concentration of 50 µg/vial. This antibody has not been prepared by aseptic techniques.

RECONSTITUTION
Reconstitute the lyophilized material with 1 mL of deionized or distilled water.

IMMUNOGEN
Bacterially derived fusion protein containing the human papillomavirus type 16 early protein E7 (HPV16-E7) open reading frame (ORF).[1]

CLONE	ISOTYPE	TOTAL PROTEIN CONCENTRATION	ANTIBODY CONCENTRATION
8C9	Mouse IgG_1	??? g/L	??? mg/L

SPECIFICITY
In Western blots, against the HPV16-E7 bacterially derived fusion protein, the antibody detects a 52 kD band which is composed of the HPV16-E7 protein of M_r 15 kD and the trpE protein of M_r 37 kD.[(1)] Positive reactivity for the 15 kD HPV16-E7 expressed protein was shown with CaSki (HPV16 DNA containing cell line) cells by Western blot. The antibody also has been shown to immunoprecipitate and immunocytochemically stain the HPV16-E7 expressed protein from the CaSki cell line.[(2)]

Negative reactivity by Western blot was shown with a series of other HPV fusion proteins including: HPV16-E2, HPV16-E4, HPV16-E6, HPV18-E7, HPV6-E7, HPV6-E4, and HPV6-L1. The antibody failed to react with HeLa (HPV18 DNA containing cell line) cells and other HPV DNA negative cervical carcinoma cell lines, HT-3 and C-33A, by Western blot, by immunoprecipitation or by immunocytochemical staining analyses.[(2)]

BACKGROUND
Specific types of HPV have been shown to be associated with human cervical cancer.[(4,5,6)] One of these types, HPV16, has been shown to be present in the majority of cervical dysplasias and squamous cell carcinomas studied.[(7,8)] Research has also shown that the expression of the HPV16-E7 gene is both sufficient and necessary for cellular transformation *in vitro*.[(9, 10)]

The immunological detection of HPV types in human samples has been hindered by the lack of an *in vitro* culture system for the propagation of viruses and viral antigens. Although antibodies that were generated against the bovine papillomavirus capsid antigen (BPV-L1 ORF) are cross-reactive to HPV**,** these antibodies are of limited use because they do not discriminate among HPV types and have limited sensitivity as compared to standard light microscopic criteria for HPV infection.[(11)] With bacterial expression systems, fusion proteins of HPV-encoded gene products can be generated and these, in turn, used to develop HPV type specific antibodies.[(1,12)] The use of these type specific antibodies to study HPV protein expression may expand understanding of the role of HPV proteins in the pathogenesis of condylomas, dysplasias, and carcinomas of the genital tract.[(1 2)]

USAGE
These are recommended dilutions. Optimal dilutions for any procedure should be determined by the user.

Immunohistochemistry - 1:2
Immunoprecipitation - 1:10

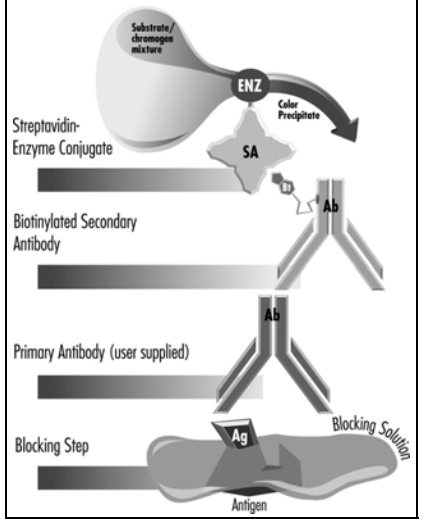
Western blotting - 1:100

The correlation of the immunohistochemical and immunocytochemical performance of this antibody with HPV DNA hybridization techniques on human samples has not been established.

IMMUNOHISTOCHEMICAL (IHC) STAINING

Historically, specific demonstration of antigens have been done with fluorescent or enzymatic secondary antibody conjugates, PAP or APAAP, and ABC. Currently, the LAB-SA[13] method (also known as the Streptavidin-Peroxidase (SP) and Streptavidin-Alkaline Phosphatase (SAP) methods,[14] see Figure 1) is preferred in the immunohistochemistry laboratory due to its higher sensitivity, low background, ease-of-use, and shorter protocol times.

Figure 1 - **LAB-SA Method**



Tissue specimens should be pre-incubated with blocking reagents to reduce nonspecific background staining. Specific staining is accomplished by localizing the HPV16-E7 antigens with this HPV16-E7 monoclonal antibody. The antigen/antibody complex is then identified using the LAB-SA detection method, or other immunodetection system. The immunodetection method is supplied by the user. When using the LAB-SA method, a biotinylated secondary antibody will bind to the primary antibody that is complexed with the antigen. A streptavidin-enzyme conjugate is then added which binds to the biotinylated secondary antibody. A substrate/chromogen solution is then added that forms a colored deposit in the presence of the enzyme which is complexed to the antigen. The location of the antigen is then revealed by the presence of the colored deposit that forms around it.

The LAB-SA method is illustrated in Figure 1. The clinical interpretation of staining or its absence should be complemented by positive and negative controls (see Controls Section), and be performed and interpreted by qualified individuals.

Materials Not Provided	**Catalog No.**		**Catalog No.**
AEC (aminoethyl carbazole)	Invitrogen, 00-2007	Histomount™ (mounting medium for DAB)	Invitrogen, 00-8030
Antibody Diluent	Invitrogen, 00-3118		
Bovine Serum Albumin (BSA)	Sigma, A 3424	Mayer's hematoxylin	Invitrogen, 00-8011
DAB (3,3'diaminobenzidine)	Invitrogen, 88-2014		
GVA (mounting medium for AEC)	Invitrogen, 00-8000	PBS (0.01 M PBS)	Invitrogen, 00-3000
HistoGrip™	Invitrogen, 00-8050	Sodium Azide (NaN_3)	Sigma, S 2002

Coverslips, Ethyl alcohol (EtOH, reagent grade) , Humidifying chamber, Microscope, Microscope slides, , Normal serum (non-immune from secondary host), Timer, Staining jars, Xylene.

IMMUNOHISTOCHEMICAL STAINING PROCEDURE

A. Tissue Section Preparation

This procedure is for routinely fixed, paraffin-embedded tissue mounted onto a slide. Use adhesive to bond tissue to slide (i.e., HistoGrip™, or poly-L-lysine coated slides).

1.	Deparaffinize tissue before staining.	
2.	If necessary, block endogenous biotin activity	
3.	If necessary quench endogenous enzyme activity.	
4.	Block in normal serum (Reagent 1A, from Histostain™-SP kit, Ready-to-use)	10 min.

B. Primary Antibody Incubation

1.	Dilute HPV16-E7 antibody 1:2 with diluent (10 mM PBS with 1% BSA and 0.1% $NaN3_3$, or Invitrogen Antibody Diluent Cat. No. 00-3118)	
2.	Incubate with diluted HPV16-E7 antibody solution	30 min.
3.	Wash in PBS bath	3 changes; 2 min. each

C. Antibody Detection,

USING A HISTOSTAIN™-SP KIT

Positive staining of HPV16-E7 in formalin-fixed, paraffin-embedded tissue sections has been achieved at an antibody dilution of 1:2 using a Invitrogen Histostain™-SP kit with the following recommended protocol. All steps are performed at room temperature with this protocol unless otherwise indicated.

1.	Incubate with biotinylated anti- mouse antibody (Reagent 1B, Ready-to-use)	10 min.
2.	Wash in PBS	3 changes; 2 min. each
3.	Incubate with streptavidin- HRP conjugate (Reagent 2, Ready-to-use)	10 min.
4.	Wash in PBS	3 changes; 2 min. each
5.	Develop with chromogen (Reagent 3A, 3B, and 3C. One drop of each into 1 ml of distilled H_2O)	5 - 10 min.
6.	Rinse in distilled water	3 min.
7.	Counter staining and mounting - follow kit instructions.	

USING OTHER IMMUNODETECTION SYSTEMS

Where a commercial secondary antibody detection kit (for murine antibodies) is used, follow the manufacturer's instructions. If a commercial detection kit is not utilized, a recommended procedure is as follows:

1.	Incubate with anti-mouse secondary antibody	30 min.
2.	Wash in PBS	3 times; 2 min. each
3.	Incubate with enzyme reagent (for example, ABC or PAP)	30 min.
4.	Wash in PBS	3 times; 2 min. each
5.	Develop chromogen	5-10 min.
6.	Wash in distilled water	3 min.

D. Tissue Counterstaining and Preservation

Counterstain (optional) according to manufacturer's instructions. Mount slides. Note: stability of positive staining depends on the compatibility and nature of the counterstain, mounting medium, and state of tissue (hydrated or dehydrated). Fading of stain on slides over time may occur.

CONTROLS

As in any laboratory procedure the use of appropriate controls is essential. A **Positive Tissue Control** consisting of known areas of positive and negative staining for HPV 16-E7 should be included. A reagent control (**Negative Control-1**) should also be run in which the primary antibody step is replaced with non-specific or non-immune mouse IgG for each tissue in question. A tissue control (**Negative Control-2**) which is known not to exhibit HPV 16-E7 staining should also be run. The staining performance of the controls, as well as the effects of the differences in fixation, must be taken into consideration when interpreting the results by a qualified individual.