**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.

**REFERENCES**

2. Data on file at Triton Diagnostics Inc.

**TRADEMARKS**

Histogrip™, Histomount™, Histostain™, and Peroxo-Block™ are trademarks of Invitrogen.

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**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 protein containing the human papillomavirus type 16 early protein E7 (HPV16-E7) open reading frame (ORF).1

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**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, 15 kD and the ttp1 protein of M, 37 kD. 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.23

Negative reactivity by Western blot was shown with a series of other HPV fusion proteins including: HPV16-E2, HPV16-E4, HPV16-E6, HPV18-E7, HPV16-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.23

**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.19 Research has also shown that the expression of the HPV16-E7 gene is both sufficient and necessary for cellular transformation in vitro.8,10

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.11,12 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.11,12

**USAGE**

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

- Immunohistochemistry - 1:2
- Immunoprecipitation - 1:10
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, 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 times; 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 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.