HPV Cocktail Broad Spectrum (HPV-1, 6, 11, 16, 18 and 31)

Concentrated and Prediluted Cocktail Antibody
902-177-103019

BIOCARE MEDICAL

Catalog Number:	**CM 177 CK**	**PM 177 AA**	**VLTMX 177 G20**
Description:	1.0 mL, conc.	6.0 mL, RTU	20 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use
Diluent:	HPV Diluent	N/A	N/A

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

Summary and Explanation:
The HPV Cocktail Broad Spectrum antibody is a cocktail of two monoclonal antibody clones (BPV-1/1H8 + CAMVIR-1) that target the major capsid protein (L1) of multiple human papillomavirus (HPV) subtypes (1-3). BPV-1/1H8 antibody was raised against disrupted bovine papillomavirus type 1 (BPV-1) and used to identify the product of the L1 open reading frame (ORF) of BPV-1. BPV-1/1H8 was tested by ELISA, and with immunohistochemical and immunofluorescent techniques, shown to recognize HPV-1, 6, 11, 16, 18, and 31 in formalin-fixed paraffin-embedded (FFPE) biopsy specimens (4,5). The CAMVIR-1 antibody was raised against L1 of HPV subtype 16. Other HPV subtypes may also be reactive with the HPV Cocktail Broad Spectrum antibody but have not been tested.

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: BPV-1/1H8 + CAMVIR-1
Isotype: IgG + IgG2a
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: HPV Cocktail Broad Spectrum (HPV-1, 6, 11, 16, 18 and 31)
Cellular Localization: Nuclear
Positive Tissue Control: Infected cervical biopsy
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative
HPV Diluent (PD906)

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTMX177 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:
Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).

Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block (Optional): Incubate for 10-20 minutes at RT with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Secondary: Incubate for 10 minutes with Val Mouse Secondary.
Linker: Incubate for 10 minutes with Val Universal Linker.
Polymer: Incubate for 10 minutes with Val Universal Polymer.
Chromogen: Incubate for 5 minutes with Val DAB.
Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Reveal or Diva Decloaker. Refer to the Reveal or Diva Decloaker product data sheet for specific instructions.
Protein Block: Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
Technical Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Staining Protocol Recommendations (Ventana BenchMark ULTRA):
PM177 is compatible for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
- Using ***ultra*View:**
Template/Detection: ultraView DAB
Pretreatment Protocol: CC1 Standard
Primary Antibody: 44 minutes, 37°C
- Using **OptiView:**
Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 32 minutes
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 16 minutes, 36°C

Limitations:
This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

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Precautions Cont'd:

ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (7)

2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (8)

3. Microbial contamination of reagents may result in an increase in nonspecific staining.

4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

5. Do not use reagent after the expiration date printed on the vial.

6. The SDS is available upon request and is located at http://biocare.net.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

References:

1. Tomita Y, Shirasawa H, Simizu B. Expression of human papillomavirus types 6b and 16 L1 open reading frames in Escherichia coli: detection of a 56,000-dalton polypeptide containing genus-specific (common) antigens. J Virol. 1987 Aug;61(8):2389-94.

2. Cowsert LM, Lake P, Jenson AB. Topographical and conformational epitopes of bovine papillomavirus type 1 defined by monoclonal antibodies. J Natl Cancer Inst. 1987 Nov;79(5):1053-7.

3. Cowsert LM, Pilacinski WP, Jenson AB. Identification of the bovine papillomavirus L1 gene product using monoclonal antibodies. Virology. 1988 Aug;165(2):613.

4. Xiao CY, *et al.* Observations on the expression of human papillomavirus major capsid protein in HeLa cells. Cancer Cell Int. 2015 May 23;15:53.

5. McInnes E, *et al.* Intranuclear Inclusions in Renal Tubular Epithelium in Immunodeficient Mice Stain with Antibodies for Bovine Papillomavirus Type 1 L1 Protein. Vet Sci. 2015 Jun 11;2(2):84-96.

6. McLean CS, *et al.* Production and characterisation of a monoclonal antibody to human papillomavirus type 16 using recombinant vaccinia virus. J Clin Pathol. 1990 Jun;43(6):488-92.

7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."

8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

Rev: 062117