Uroplakin II + Uroplakin III

Prediluted Monoclonal Antibody Cocktail
Control Number: 901-3094-052623

BIOCARE MEDICAL

Catalog Number:	**API 3094 AA**	**VLTM 3094 G20**
Description:	6.0 mL, RTU	20 mL, RTU
Dilution:	Ready-to-use	Ready-to-use
Diluent:	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
Uroplakin II + Uroplakin III [BC21 + BC17] is a mouse monoclonal antibody cocktail intended for laboratory use in the qualitative identification of Uroplakin II and Uroplakin III proteins by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
Uroplakin II (UPII) and Uroplakin III (UPIII) are 15 kDa and 47 kDa proteins, respectively, that are key components of urothelial plaques, which enhance the permeability barrier of the urothelium (1). UPII and UPIII may be found in the urothelial surface membrane of human renal pelvis, ureter, bladder and urethra. UPII and UPIII have also been identified as sensitive and highly specific markers for urothelial carcinoma (2-5).

New mouse monoclonal antibodies to UPII, clone BC21, and UPIII, clone BC17, have been developed and evaluated for sensitivity in urothelial carcinoma and specificity versus normal and neoplastic tissues (6). In a study of 56 cases of urothelial carcinoma, UPII [BC21] and UPIII [BC17] stained 44 (79%) and 31 (55%) cases, respectively (6). With the exception of bladder and ureter, UPII and UPIII have been found to be highly specific when evaluated in various normal and neoplastic tissues, including breast, lung, colon, prostate, kidney, ovarian, liver and pancreatic cancers. As a result, the combination of UPII [BC21] and UPIII [BC17] may be a sensitive marker for urothelial carcinoma and may be valuable in the discrimination of bladder cancer from renal and prostate carcinomas. U.S. Patent 9,429,577 and patents pending.

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.

Reagent Provided:
Uroplakin II + Uroplakin III is provided as a prediluted antibody cocktail of anti-Uroplakin II and anti-Uroplakin III antibodies, in buffer with carrier protein and preservative.

Antibody	anti-Uroplakin II	anti-Uroplakin III
Clone	BC21	BC17
Source	Mouse monoclonal	Mouse monoclonal
Isotype	IgG1	IgG1
Epitope/ Antigen	Residues 36-50	Uroplakin III
Cellular Localization	Cytoplasmic & Membrane	Cytoplasmic & Membrane
Staining	Brown (DAB)	Brown (DAB)

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.
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human; others not tested
Positive Tissue Control: Urothelial carcinoma or normal bladder

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTM3094 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).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block (Optional): Incubate for 10-20 minutes with Val Background Block.
Primary Antibody: Incubate for 45 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 Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.
Protein Block (Optional): 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.

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Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011

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 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.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:
1. Wu XR, *et al.* Uroplakins in urothelial biology, function, and disease. Kidney Int. 2009 Jun; 75(11):1153-65.
2. Moll R, *et al.* Uroplakins, specific membrane proteins of urothelial umbrella cells, as histological markers of metastatic transitional cell carcinomas. Am J Pathol. 1995 Nov; 147(5):1383-97.
3. Kaufmann O, Volmerig J, Dietel M. Uroplakin III is a highly specific and moderately sensitive immunohistochemical marker for primary and metastatic urothelial carcinomas. Am J Clin Pathol. 2000 May; 113(5):683-7.
4. Olsburgh J, *et al.* Uroplakin gene expression in normal human tissues and locally advanced bladder cancer. J Pathol. 2003 Jan; 199(1):41-9.
5. Huang HY, *et al.* Persistent uroplakin expression in advanced urothelial carcinomas: implications in urothelial tumor progression and clinical outcome. Hum Pathol. 2007 Nov; 38(11):1703-13.
6. Hoang LL, *et al.* A newly developed Uroplakin II antibody with increased sensitivity in urothelial carcinoma of the bladder. Arch Pathol Lab Med. 2013; in press.
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."

References Cont'd:
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.

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