Uroplakin II

Concentrated and Prediluted Monoclonal Antibody
901-3051-040319

BIOCARE MEDICAL

Catalog Number:	ACI 3051 A, C	API 3051 AA	OAI 3051 T60	AVI 3051 KG	VLTM 3051 G20
Description:	0.1, 1.0 mL, conc.	6.0 mL, RTU	60 tests, RTU	6.0 mL, RTU	20 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Van Gogh Yellow	N/A	N/A	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
Uroplakin II [BC21] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of uroplakin II protein 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 is a 15 kDa protein component of urothelial plaques, which enhance the permeability barrier of the urothelium (1). Studies have shown Uroplakin II mRNA was expressed in bladder cancer tissues and peripheral blood of patients with primary and metastatic urothelial carcinoma of the bladder (2-4). A new mouse monoclonal Uroplakin II antibody [BC21] was developed and exhibited an increased staining sensitivity (46/59, 78%) when compared to Uroplakin III [AU1] (19/56, 34%) in cases of urothelial carcinoma of the bladder (see Performance Characteristics). With the exception of bladder and ureter, Uroplakin II [BC21] was found to be highly specific when evaluated in various normal and neoplastic tissues, including prostate cancer and renal cell carcinoma (see Performance Characteristics). Uroplakin II [BC21] is a highly specific antibody that may be useful in identifying tumors of urothelial origin.
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.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: BC21
Isotype: IgG1/kappa
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: Residues 36-50 of human Uroplakin II
Cellular Localization: Cytoplasmic and membrane
Positive Tissue Control: Normal bladder or urothelial carcinoma of the bladder

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

For AVI3051KG:
Uroplakin II (AVI3051G) 1 x 6ml
V-Blocker (BRI4001G) 1 x 6ml

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):
VLTM3051 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-Lo 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 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 Decloaker. Refer to the Reveal 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.

Protocol Recommendations (ONCORE™ Automated Slide Staining System):
OAI3051 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: Uroplakin II
Protocol Template (Description): Ms HRP Template 1
Dewaxing (DS Option): DS2
Antigen Retrieval (AR Option): AR2, low pH; 101°C
Reagent Name, Time, Temp.: Uroplakin II, 30 min., 25°C

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Protocol Recommendations (Ventana BenchMark XT / ULTRA):
AVI3051 is intended for use with the BenchMark XT / ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using ***ultra*View on XT / ULTRA:**

Template/Detection: ultraView DAB
Pretreatment Protocol: CC1 Mild
Primary Antibody: 32 minutes, 37°C
ultraBlock (V-Blocker BRI4001): Incubate for 4 minutes (with appropriate Option # registered by user)
V-Blocker is recommended to be applied prior to any detection system.

- Using **OptiView on ULTRA:**

Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 32 minutes
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 8 minutes, 36°C

Performance Characteristics:
Sensitivity, specificity and cross-reactivity were determined by staining with MACH 4 Universal HRP-Polymer Detection. See Tables 1 and 2 for expected results.

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) (5)
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 into contact with sensitive areas, wash with copious amounts of water. (6)
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. Wu X, *et al.* Uroplakin II as a promising marker for molecular diagnosis of nodal metastases from bladder cancer: comparison with cytokeratin 20. J Urol. 2005 Dec; 174(6):2138-42.
3. Lu JJ, *et al.* Detection of circulating cancer cells by reverse transcription-polymerase chain reaction for uroplakin II in peripheral blood of patients with urothelial cancer. Clin Cancer Res. 2000 Aug; 6(8):3166-71.
4. Li SM, *et al.* Detection of circulating uroplakin-positive cells in patients with transitional cell carcinoma of the bladder. J Urol. 1999 Sep; 162(3 Pt 1):931-5.
5. 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."
6. 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.

Table 1: Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded neoplastic tissues.

Tissue Types	# Positive / Total Cases
Bladder cancer	46/59*
Prostate cancer	1/88**
Lung cancer	0/20
Kidney cancer (various phenotypes)	3/75***
Colon cancer	0/63
Brain cancer	0/13
Lymphoma	0/25
Melanoma	0/19
Ovarian cancer	0/11
Seminoma	0/14
Breast cancer	0/74
Adrenal gland cancer	0/2
Thyroid cancer	0/2
Pancreas cancer (various phenotypes)	0/10
Head & neck cancer (various phenotypes)	0/10
Soft tissue cancer (various phenotypes)	0/10
Liver cancer (various phenotype)	0/10
Cervix cancer (various phenotypes)	0/10

* For comparison, Uroplakin III [Clone AU1] stained 19/56 cases of bladder cancer.
** 1 positive case, which may be metastatic bladder cancer that has spread to prostate
*** 3 positive cases, which are transitional cell carcinomas from upper ureters

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Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# Positive / Total Cases	Tissue	# Positive / Total Cases
Adrenal gland	0/3	Pancreas	0/5
Bladder	5/7	Parathyroid	0/1
Bone marrow	0/1	Pituitary gland	0/2
Eye	0/2	Placenta	0/3
Breast	0/3	Prostate	0/5
Cerebellum	0/3	Skin	0/2
Cerebral cortex	0/3	Spinal cord	0/2
Fallopian tube	0/3	Spleen	0/2
GI-Esophagus	0/3	Striated muscle	0/4
GI-Stomach	0/3	Testis	0/3
GI-Small intestine	0/3	Thymus	0/3
GI-Colon	0/3	Thyroid	0/4
GI-Rectum	0/3	Tonsil	0/3
Heart	0/3	Ureter	3/3
Kidney	0/16	Uterus-cervix	0/3
Liver	0/5	Uterus-endometrium	0/3
Lung	0/3	Tongue	0/1
Ovary	0/3	Epiglottis	0/1
		Blood vessel and adipose tissue	0/1

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