Immunohistochemistry (formalin-embedded tissues) is a method used in pathology to detect the presence of antigens within tissues. This technique is particularly useful in tissue diagnostics and research. The principle of the immunohistochemical process is to employ a highly specific antibody that recognizes an antigen of interest. This antibody is used to identify the antigen within the tissue, allowing for the visualization of its location and extent.

### 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. 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).

### 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

### Clones:
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 2°C to 8°C. 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 2°C to 8°C.

### Protocol Recommendations (VALENT® Automated Slide Staining Platform):

#### Peroxidase Block:
Block for 5 minutes with Peroxidased 1.

#### 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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**Uroplakin II**
Concentrated and Prediluted Monoclonal Antibody
901-3051-040319

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**BioCare Medical**
60 Berry Drive
Pacheco, CA 94553
USA

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

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**BioCare Medical**
Prinsessegracht 20
2514 AP The Hague
The Netherlands
Uroplakin II
Concentrated and Prediluted Monoclonal Antibody
901-3051-040319

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 ultraView on XT / ULTRA:
  
  **Template/Detection:** ultraView DAB
  
  **Pretreatment Protocol:** CC1 Mild
  
  **Primary Antibody:** 32 minutes, 37°C
  
  **ultraBlock (V-Blocker BR14001):** 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 fixed, 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:


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 (NaN₃) 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 nonspecific 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:


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

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

* 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

References:


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

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