

GATA-3 + Uroplakin II

Prediluted Antibody Cocktail
901-3173-042618

BIOCARE
M E D I C A L

Catalog Number:	API 3173 AA	AVI 3173 G
Description:	6.0 ml, prediluted	6.0 ml, prediluted
Dilution:	Ready-to-use	Ready-to-use
Diluent:	N/A	N/A

Intended Use:

For In Vitro Diagnostic Use

GATA-3 + Uroplakin II is a cocktail of mouse monoclonal antibodies that is intended for laboratory use in the qualitative identification of GATA-3 and uroplakin II 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:

Distinguishing between invasive urothelial carcinoma (UC) from other genitourinary malignancies, such as prostatic and renal carcinomas, can be difficult. Several new markers such as GATA binding protein 3 (GATA-3), S100P, and uroplakin III have been developed for this differentiation (1-7). More recently uroplakin II has been introduced (1-7).

GATA-3 is a transcription factor and belongs to a distinct family of tumor suppressor genes. It is involved in human cancer cell growth and differentiation, and plays an important role in cell proliferation and apoptosis. Recent studies have found GATA-3 to be a sensitive and specific marker for UC. The level of GATA-3 expression was seen to be an independent factor predicting cancer recurrence (6,7).

Uroplakins are markers of terminally differentiated bladder urothelia. Uroplakin II (UPII) is a newly described sensitive marker for UC (1-5). UPII has been shown to be a more sensitive immunohistochemical (IHC) marker than uroplakin III in UC (1,2). UPII has also been used in the diagnosis for UC using a panel that includes GATA-3 and p40 (4,5). The combination of GATA-3 (nuclear) and UPII (cytoplasmic/membrane) in a single color format increased both specificity and sensitivity in a single section (4,5). UPII has been shown to be virtually 100% specific for UC (1-3). GATA-3 increases the overall specificity when compared to UPII alone and it does not stain prostate adenocarcinoma or renal cell carcinoma (4,5). Thus, two antigens, GATA-3 (nuclear) and UPII (cytoplasmic/membrane), can be recognized and displayed on the same section by a single color format.

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. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

Reagent Provided:

GATA-3 + Uroplakin II is provided as a prediluted antibody cocktail of anti-GATA-3 and anti-Uroplakin II antibodies in buffer with carrier protein and preservative.

Antibody	anti-GATA-3	anti-Uroplakin II
Clone	L50-823	BC21
Source	Mouse monoclonal	Mouse monoclonal
Isotype	IgG1/k	IgG1/k
Epitope/ Antigen	GATA-3 peptide	Residues 36-50
Cellular Localization	Nuclear	Cytoplasmic/membrane
Staining	Brown (DAB)	Brown (DAB)

Storage and Stability:

Store at 2°C to 8°C. Do not use reagent after the expiration date printed on the vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity: Human; others not tested

Positive Tissue Control: Bladder cancer

Protocol Recommendations (intelliPATH and manual use):

Peroxide Block: Block for 5 minutes with Biocare's Peroxidized 1.

Pre-treatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's 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 Biocare's Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Protocol Recommendations (Ventana BenchMark ULTRA Slide Staining System):

AVI3173 is intended for use with the Ventana BenchMark ULTRA Slide Staining System. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using **ultraView:**

Template/Detection: ultraView DAB

Pretreatment Protocol: CC1 Standard

Primary Antibody: 32 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

Technical Note:

This antibody, for intelliPATH and manual use, has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a

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Limitations Cont'd:

qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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 (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) (8)
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. (9)
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. Hoang LL, Tacha DE, Qi W, Yu C, Bremer RE, Chu J, Haas TS, Cheng L. A newly developed uroplakin II antibody with increased sensitivity in urothelial carcinoma of the bladder. Arch Pathol Lab Med. 2014 Jul;138(7):943-9.
2. Tian W, Guner G, Miyamoto H, Cimino-Mathews A, Gonzalez-Roibon N, Argani P, Li X, Sharma R, Subhawong AP, Rezaei K, Bivalacqua TJ, Epstein JI, Bishop JA, Netto GJ. Utility of uroplakin II expression as a marker of urothelial carcinoma. Am J Clin Pathol. 2014 Dec;142(6):864-71.
3. Li W, Liang Y, Deavers MT, Kamat AM, Matin SF, Dinney CP, Czerniak B, Guo CC. Uroplakin II is a more sensitive immunohistochemical marker than uroplakin III in urothelial carcinoma and its variants. Am J Clin Pathol. 2014 Dec;142(6):864-71.
4. Hoang LL, Tacha D, Bremer RE, Haas TS, Cheng L. Uroplakin II (UPII), GATA3, and p40 are Highly Sensitive Markers for the Differential Diagnosis of Invasive Urothelial Carcinoma. Appl Immunohistochem Mol Morphol. 2015 Nov-Dec;23(10):711-6.
5. Leivo MZ, Elson PJ, Tacha DE, Delahunt B, Hansel DE. A combination of p40, GATA-3 and uroplakin II shows utility in the diagnosis and prognosis of muscle-invasive urothelial carcinoma. Pathology. 2016 Oct;48(6):543-9.
6. Mohammed KH, Siddiqui MT, Cohen C. GATA3 immunohistochemical expression in invasive urothelial carcinoma. Urol Oncol. 2016 Oct;34(10):432.

References Cont'd:

7. Miyamoto H, Izumi K, Yao JL, Li Y, Yang Q, McMahon LA, Gonzalez-Roibon N, Hicks DG, Tacha D, Netto GJ. GATA binding protein 3 is down-regulated in bladder cancer yet strong expression is an independent predictor of poor prognosis in invasive tumor. Hum Pathol. 2012 Nov;43(11):2033-40.
8. 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."
9. 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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