

Uro-2™ (CK20 + p53)
Prediluted Multiplex Cocktail (4-Step)
Control Number: 902-3001DS-090717

Catalog Number: APR 3001DS AA

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

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

Summary and Explanation:

Uro-2 (CK20 + p53) is a primary antibody cocktail for the multiplex IHC identification of CK20 and p53 proteins in bladder. Studies have shown that in normal urothelium, the superficial umbrella cell layer shows reactivity for CK20 only; whereas, p53 nuclear staining is absent to focal. For urothelium with reactive atypia, particularly in cases with marked atypia, CK20 and p53 staining remain identical to those seen in normal urothelium. In cases of CIS, diffuse, strong cytoplasmic reactivity for CK20 and diffuse nuclear reactivity for p53 is observed throughout the urothelium. Most high-grade dysplasia stain with p53 when compared to low-grade dysplasia.

Principle of Procedure:

This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Source: Mouse monoclonal and Rabbit monoclonal

Species Reactivity: Human; others not tested

Clone: Ks20.8 + EP9 (previously known as Y5)

Isotype: IgG2a + IgG

Epitope/Antigen: CK20 and p53 protein

Cellular Localization:

CK20 (Cytoplasmic): Brown

p53 (Nuclear): Red

[Optional] CD44 (Cell membrane, cytoplasmic): Purple (see optional protocol)

Positive Control: p53 positive bladder or colon cancers

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

Uro-2™ (CK20+p53) 6ml

Buffer with protein carrier and preservative

Storage and Stability:

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

Staining Protocol Recommendations:

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

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:

Heat Retrieval Method:

Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water; alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

Protein Block (Optional): Incubate for 10 minutes at RT with Biocare's Background Punisher.

Primary Antibody Cocktail: (CK20 + p53): Incubate for 30 minutes at RT.

Double Stain Detection: Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2.

Staining Protocol Recommendations Cont'd:

Chromogen (1): Incubate for 5 minutes at RT with Biocare's Betazoid DAB.

Chromogen (2): Incubate for 5-7 minutes at RT with Biocare's Warp Red.

(Optional) Protocol for additional staining with CD44 (APR380AA): Staining protocol for reactive atypia:

Rinse in DI Water.

Wash in TBS Wash Buffer.

Denaturing Solution: Incubate for 3 minutes with Denaturing Solution A and B (BRR001D) diluted at a ratio of 1:4.

Primary Antibody: CD44: Incubate for 30 minutes at RT.

Detection: Incubate with Biocare's MACH 2 HRP for 30 minutes.

Chromogen (3): Incubate with Biocare's Bajoran Purple for 7 minutes. Rinse in deionized water.

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

Technical Note:

This antibody has been standardized with Biocare's MACH 2 Double Stain 2. It can also be used on an automated staining system. Use TBS buffer for washing steps.

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 (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) (4)
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. (5)
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. Russo S, *et al.* A useful panel in proliferation urothelial lesions: an analysis of cytokeratin 20, p53, CD44 and Ki-67 antigens. *Pathologica*. 2007 Apr; 99(2):46-9.
2. McKenney JK, *et al.* Discriminatory immunohistochemical staining of urothelial carcinoma in situ and non-neoplastic urothelium: an analysis of cytokeratin 20, p53 and CD44 antigens. *Am J Surg Pathol*. 2001 Aug; 25(8):1074-8.
3. Sun W, Zhang PL, Herrera GA. p53 protein and Ki-67 overexpression in urothelial dysplasia of bladder. *Appl Immunohistochem Mol Morphol*. 2002 Dec; 10(4):327-31.
4. 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."
5. 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.

Produced using Abcam's RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.