URO-3 Triple Stain (CD44 + p53) with CK20

Prediluted Triple Stain Antibody Cocktail
901-370TS-031518

BIOCARE MEDICAL

Catalog Number:	**PM 370 TS AA**
Description:	6.0 ml, prediluted
Dilution:	Ready-to-use
Diluent:	N/A

Intended Use:
For In Vitro Diagnostic Use
URO-3 Triple Stain (CD44 + p53) with CK20 is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of CD44, p53 and CK20 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:
URO-3 Triple Stain (CD44 + p53) with CK20 is a primary antibody cocktail of CD44 and p53 antibodies, plus a CK20 primary antibody for the multiplex IHC identification of CD44, p53 and CK20 proteins in bladder. Studies have shown that in normal urothelium, the superficial umbrella cell layer shows reactivity for CK20 only, whereas CD44 staining is limited to the basal and parabasal urothelial cells and p53 nuclear staining is absent to focal. For urothelium with reactive atypia, particularly in cases with marked atypia, CD44 shows increased reactivity in all layers of the urothelium and is often absent in neoplastic cells. CK20 and p53 staining remain identical to those seen in normal urothelium. In cases of CIS, diffuse cytoplasmic reactivity for CK20, and in most cases, diffuse nuclear reactivity for p53 is observed throughout the urothelium.

Principle of Procedure:
A three-color Multiplex IHC stain can be accomplished in seven major steps. The initial step consists of an antibody cocktail with at least one mouse and one rabbit antibody. This cocktail is applied to the tissue and will bind with two or more target antigens. A multiplex detection cocktail of horseradish peroxidase (HRP) and alkaline phosphatase (AP) conjugated secondary antibodies is applied. The third step consists of the addition of Betazoid DAB, which produces a brown chromogenic product, through an HRP-catalyzed process. The fourth step consists of the addition of an alkaline phosphatase chromogen (Ferangi Blue or Fast Red), which produces a blue or red chromogenic product, through an AP-catalyzed process. A denaturing solution is then used to remove the primary antibodies applied in the first step. In the fifth and sixth steps, a final primary antibody is applied, followed by an AP conjugated secondary antibody. In the final step, Fast Red or Ferangi Blue is applied, which produces a red or blue chromogenic product, through an AP-catalyzed process.

Source: Mouse monoclonal + Rabbit monoclonal + Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: BC8 (CD44)
EP9 (previously known as Y5) (p53)
Ks20.8 (CK20)
Isotype: IgG1, IgG and IgG2a
Epitope/Antigen: CD44 + p53 and CK20
Cellular Localization:
CD44 (Blue or Red): Cytoplasmic
p53 (Brown): Nuclear
CK20 (Red or Blue): Cytoplasmic
Positive Tissue Control: Some p53-positive bladder and colon carcinomas

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: CD44+p53 (PM373DSAA) 6ml
CK20 (PM062AA) 6ml
Storage and Stability:
Store at 2ºC to 8ºC. 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.

Protocol Recommendations:
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.
Pretreatment: 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.

- Method 1: Three-color stain: CD44 (Blue), p53 (Brown), CK20 (Red):
Primary Antibody Cocktail: (CD44 + p53): Incubate for 30 minutes.
Secondary Antibody: MACH 2 Double Stain 1 for 30 minutes.
Chromogen (1): Incubate for 5 minutes with Biocare's Betazoid DAB.
Chromogen (2): Incubate for 5 minutes with Biocare's Ferangi Blue.
Denaturing Solution: Incubate for 3 minutes with Denaturing Solutions A and B (DNS001) diluted at a ratio of 1:4.
Primary Antibody: (CK20): Incubate for 20-30 minutes.
Secondary Antibody: MACH 2 Mouse AP for 20-30 minutes.
Chromogen (3): Incubate with Biocare's Vulcan Fast Red (10-15 min) or Warp Red (5-7 min). Rinse in deionized water.

- Method 2: Three-color stain: CD44 (Red), p53 (Brown), CK20 (Blue):
Primary Antibody Cocktail: (CD44 + p53): Incubate for 30 minutes.
Secondary Antibody: MACH 2 Double Stain 1 for 30 minutes.
Chromogen (1): Incubate for 5 minutes with Biocare's Betazoid DAB.
Chromogen (2): Incubate with Biocare's Vulcan Fast Red (10-15 min) or Warp Red (5-7 min).
Denaturing Solution: Incubate for 3 minutes with Denaturing Solutions A and B diluted at a ratio of 1:4.
Primary Antibody: (CK20): Incubate for 20-30 minutes.
Secondary Antibody: MACH 2 Mouse AP for 20-30 minutes.
Chromogen (3): Incubate for 5 minutes with Biocare's Ferangi Blue. Rinse in deionized water.

Counterstain/Mounting:
Weigert's Hematoxylin or light hematoxylin without bluing is recommended. Rinse in deionized water. Dry slides for 10-20 minutes at 60 degrees and mount with Biocare's EcoMount (non-xylene based mounting medium).

Technical Note:
This procedure can also be performed on an automated staining system, including Biocare's intelliPATH. Recommended chromogen systems for the intelliPATH are: intelliPATH DAB, intelliPATH Ferangi Blue, intelliPATH Fast Red, and intelliPATH Warp Red. Use TBS buffer for all washing steps. Ferangi Blue is soluble in alcohol and xylene.

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

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

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

Limitations Cont'd:
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 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 (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) (3)
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. (4)
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. Russo S, *et al.* A useful panel in proliferative 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. 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."
4. 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.

p53 was 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.

Biocare Medical
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Pacheco, CA 94553
USA

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