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 5-10 minutes at RT with Biocare's Background Puisher.

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 Betazoied DAB.

Chromogen (2): Incubate for 5 minutes with Biocare's Ferangi Blue.

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 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 Betazoied 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.

Performance Characteristics:

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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:

Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about tissue controls.
Precautions:
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)
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. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

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.

Limitations and Warranty:
There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

References: