



PIN-4™ Cocktail (CK5 + CK14 + p63 + P504S)

ISO
9001:2000
CERTIFIED

Prediluted Double Stain Antibody (4-Step)

Control Number: 902-225DS-102309

Catalog Number: PPM 225 DS AA, H, L

Description: 6.0, 25, 100 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For Research Use Only. Not for use in diagnostic procedures

Summary and Explanation:

In normal epithelia, HMW Cytokeratins (CK5 and CK14) stain basal epithelia, in the prostate gland.

p63 is detected in prostate basal epithelial nuclei in normal prostate, however, is negative in malignant tumors of the prostate gland. Thus p63 is useful as a differential marker for benign and malignant tumors of prostate gland and can be useful as a negative marker.

Expression of P504S protein is found in prostatic adenocarcinoma, but not in benign prostatic tissue. It has also been found to stain premalignant lesions of the prostate: high-grade prostatic intraepithelial neoplasia (PIN) and atypical adenomatous hyperplasia. P504S can be used as a positive marker for PIN. It will be useful to confirm the diagnosis of small focus of prostate carcinoma in needle biopsies.

The combination of P504S + HMW CKs + p63 (PIN-4™ Cocktail) may be extremely useful for diagnosing prostatic intraepithelial neoplasia, especially in difficult cases, and in cases with limited tissues. P504S stains cytoplasm in prostate adenocarcinoma and atypical adenomatous hyperplasia, and p63 and HMW CKs stain normal (negative marker) and benign prostate glands.

Source: Mouse monoclonal and Rabbit polyclonal

Species Reactivity: Human; others not tested.

Clone: XM26 + LL002 + BC4A4 + N/A

Isotype: IgG1/kappa + IgG3 + IgG2a/kappa + N/A

Epitope/Antigen: CK5 + CK14 + p63 + P504S

Cellular Localization: P504S: cytoplasmic (red), HMW CKs: cytoplasmic (brown)
p63: nuclear (brown)

Positive Control: Prostatic intraepithelial neoplasia

Normal Tissue: Prostate

Abnormal Tissue: PIN and prostate cancer

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations

Peroxide Block:

Block for 5 minutes with BIOCARE's PEROXIDAZED 1.

Pretreatment Solution (recommended): Reveal or 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 20 minutes then wash in distilled water.

Protein Block:

Incubate for 10-15 minutes at RT with BIOCARE's Background Sniper.

Primary Antibody: Incubate for 30-45 minutes at RT.

Double Stain Detection:

Incubate for 30 minutes at RT using BIOCARE's Double Stain Kit #2.

Chromogen (1): Incubate for 5 minutes at RT when using BIOCARE's Betazoid DAB.

Chromogen (2):

Incubate for 10 minutes at RT with BIOCARE's Vulcan Fast Red.

Counterstain:

Rinse with deionized water. Incubate for 30-60 seconds with Tacha's Automated Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute.

Technical Note:

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

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:

1. Tacha DE, Miller RT. Use of p63/P504S monoclonal antibody cocktail in immunohistochemical staining of prostate tissue. Appl Immunohistochem Mol Morphol. 2004 Mar;12(1):75-8. Biocare Medical, Walnut Creek, California.
2. Signoretti S, Waltregny D et al. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 2000 Dec; 157(6):1769-75.

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Prediluted Double Stain Antibody (4-Step)

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3. Beach R, Gown AM et al. P504S immunohistochemical detection in 405 prostatic specimens including 376 18-gauge needle biopsies. *Am J Surg Pathol* 2002 Dec;26(12):1588-96
4. Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, Ewing CM, Platz EA, Ferdinandusse S, Wanders RJ, Trent JM, Isaacs WB, De Marzo AM. Alpha-methylacyl-CoA racemase: a new molecular marker for prostate cancer. *Cancer Res* 2002 Apr 15;62(8):2220-6.
5. Wang Y et al. Cell differentiation lineage in the prostate. *Differentiation*. 2001 Oct;68(4-5):270-9.
6. Tokar EJ et al. Stem/progenitor and intermediate cell types and the origin of human prostate cancer. *Differentiation*. 2005 Dec;73(9-10):463-73.
7. Collins et al. Identification and isolation of human prostate epithelial stem cells based on alpha(2)beta(1)-integrin expression. *J Cell Sci*.2001 Nov;114(Pt 21):3865-72.
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. National Committee for Clinical Laboratory Standards(NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991;7(9). Order code M29-P.

PIN-4™ Cocktail (CK5 + CK14 + p63 + P504S)

Prediluted Mouse Monoclonal and Rabbit Polyclonal Double Stain Antibody (4-Step)

Control Number: 902-225DSIP-102309

Catalog Number: IP 225DS G10
IP 225DS G20
Description: 10 ml, prediluted
20 ml, prediluted

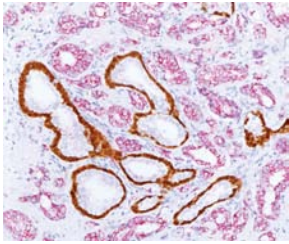
Intended Use:
For Research Use Only

Summary and Explanation:
In normal epithelia, HMW Cytokeratins (CK5 and CK14) stain basal epithelia, in the prostate gland.

p63 is detected in prostate basal epithelial nuclei in normal prostate, however, is negative in malignant tumors of the prostate gland. Thus p63 is useful as a differential marker for benign and malignant tumors of prostate gland and can be useful as a negative marker.

Expression of P504S protein is found in prostatic adenocarcinoma, but not in benign prostatic tissue. It has also been found to stain premalignant lesions of the prostate: high-grade prostatic intraepithelial neoplasia (PIN) and atypical adenomatous hyperplasia. P504S can be used as a positive marker for PIN. It will be useful to confirm the diagnosis of small focus of prostate carcinoma in needle biopsies.

The combination of P504S + HMW CKs + p63 (PIN-4™ Cocktail) may be extremely useful for diagnosing prostatic intraepithelial neoplasia, especially in difficult cases, and in cases with limited tissues. P504S stains cytoplasm in prostate adenocarcinoma and atypical adenomatous hyperplasia, and p63 and HMW CKs stain normal (negative marker) and benign prostate glands.



Prostate cancer stained with PIN-4.

Source: Mouse monoclonal and Rabbit polyclonal
Species Reactivity: Human; others not tested.
Clone: XM26 + LL002 + BC4A4 + N/A
Isotype: IgG1/kappa + IgG3 + IgG2a/kappa + N/A
Antibody Category: Double Stain, prostate cancer
Epitope/Antigen: CK5 + CK14 + p63 + P504S
Cellular Localization: P504S:cytoplasmic (red), HMW CKs: cytoplasmic (brown)
Positive Control: Prostatic intraepithelial neoplasia
Normal Tissue: Prostate
Abnormal Tissue: PIN and prostate cancer
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations

Peroxide Block:
Block for 5 minutes at RT.
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 20 minutes then wash in distilled water.

Primary Antibody:
Incubate for 30 minutes at RT.

Double Stain Detection:
Incubate for 15-30 minutes at RT using BIOCARE's Multiplex Stain Kit 2.

Chromogen (1):
Incubate for 5 minutes at RT with DAB.

Chromogen (2):
Incubate for 15-20 minutes at RT with Fast Red.

Counterstain:
1. Rinse with deionized water. 2. Incubate for 5 minutes with Hematoxylin. 3. Rinse with TBS Buffer for 1 minute followed by a rinse with deionized water.

Technical Note:
This antibody cocktail has been standardized with BIOCARE's Multiplex Stain Kit 2. Use TBS buffer for washing steps.

Performance Characteristics:
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