Prostate Cocktail (CK5 + CK14 + p63)

Prediluted Antibody Cocktail
901-3206-100517

BIOCARE MEDICAL

VP Echelon™ Series

Catalog Number: **AVI 3206 G**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Intended Use:
For In Vitro Diagnostic Use
Prostate Cocktail (CK5 + CK14 + p63) is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of CK5, CK14 and p63 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:
CK5 and CK14 are high molecular weight cytokeratins expressed in a variety of normal and neoplastic epithelial tissues (1). In prostate tissue, mRNA for CK5 and CK14 has been detected in the basal cells of normal glands and prostatic intraepithelial neoplasia (PIN), a precursor lesion to prostatic adenocarcinoma; however, expression of CK5 or CK14 was not identified in invasive prostatic adenocarcinoma (2).
p63, a homolog of the tumor suppressor p53, has been identified in proliferating basal cells in the epithelial layers of a variety of tissues, including epidermis, cervix, urothelium and prostate (3). p63 was detected in nuclei of the basal epithelium in normal prostate glands; however, it was not expressed in malignant tumors of the prostate (4).

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process.

A sequential double stain is used for the simultaneous detection of multiple antigens within one tissue section. A primary antibody is applied to the tissue, followed by a horseradish peroxidase (HRP) detection system. A denaturing step is required to eliminate cross-reactivity from the application of the second detection system. A second primary antibody is then applied, followed by an alkaline phosphatase (AP) detection system. Visualization of antigens is achieved with DAB and Red chromogens.

For single stains, the initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

Reagent Provided:
Prostate Cocktail (CK5 + CK14 + p63) is provided as a prediluted antibody cocktail of anti-CK5, anti-CK14, and anti-p63 antibodies, in buffer with carrier protein and preservative.

Antibody	anti-CK5	anti-CK14	anti-p63
Clone	XM26	LL002	4A4
Source	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal
Isotype	IgG1/kappa	IgG3	IgG2a/kappa
Epitope/ Antigen	CK5	CK14	p63
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear
Staining	DAB	DAB	DAB

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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity: Human

Positive Tissue Control: Normal prostate

Protocol Recommendations (Ventana BenchMark ULTRA):
Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Template: U IHC DS uDAB-uRed Template or U *ultra*View DAB Template
Pretreatment Protocol: ULTRA CC1 Standard (64 min) at 95°C
Primary Antibody: Incubate for 32 minutes at 37°C
ultraBlock (V-Blocker BRI4001): Incubate for 4 minutes (with appropriate Option # registered by user)
V-Blocker is highly recommended to be applied prior to any detection system.
Detection: *ultra*View DAB Detection

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 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

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Precautions Cont'd:
volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (11)
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. (12)
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:
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2. Yang Y, *et al.* differential expression of cytokeratin mRNA and protein in normal prostate, prostatic intraepithelial neoplasia, and invasive carcinoma. Am J Pathol. 1997 Feb;150(2):693-704.
3. Yang A, *et al.* p63, a p53 homolog at 3q27–29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol Cell. 1998 Sep;2(3):305-16.
4. Signoretti S, *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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6. Beach R, *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.
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8. Wang Y, *et al.* Cell differentiation lineage in the prostate. Differentiation. 2001 Oct;68(4-5):270-9.
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10. Collins AT, *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.
11. 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."
12. 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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