

# HMW CK + p63 (Basal Cell Cocktail)

Concentrated and Prediluted Cocktail Antibody  
901-210-111320

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 210 C	1.0 mL	1:100	Van Gogh Yellow
Predilute	PM 210 AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 210 T60	60 tests	Ready-to-use	N/A
VALENT	VLTM 210 G20	20 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

HMW CK + p63 (Basal Cell Cocktail) [XM26 + LL002 + 4A4] 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:

Studies have shown in normal epithelia, HMW Cytokeratins (CK5 and CK14) stain stratified epithelia, myoepithelial cells and basal cells in the prostate gland and bronchi.

p63 is detected in prostate basal cells in normal prostate; however, it is negative in malignant tumors of the prostate gland. Thus p63 is useful as a differential marker for benign and malignant tumors of the prostate gland and can be useful as a negative marker (2).

The combination of the HMW CK Cocktail and p63 has been shown to be superior to each alone (1).

## Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** XM26 + LL002 + 4A4

**Isotype:** IgG1 + IgG3 + IgG2a

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** CK5, CK14, and p63

**Cellular Localization:** HMW CK Cocktail: Cytoplasmic, p63: Nuclear

**Positive Tissue Control:** Prostatic intraepithelial neoplasia

## 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. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

## Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM210 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Lo pH, 5X (use at 1X).

**Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.

**Protein Block:** Incubate for 10 minutes with Val Background Block.

**Primary Antibody:** Incubate for 20 minutes.

**Secondary:** Incubate for 10 minutes with Val Mouse Secondary.

**Linker:** Incubate for 10 minutes with Val Universal Linker.

**Polymer:** Incubate for 10 minutes with Val Universal Polymer.

**Chromogen:** Incubate for 5 minutes with Val DAB.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

## Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Reveal Decloaker. Refer to the Reveal Decloaker product data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.

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

**Probe:** Incubate for 10 minutes at RT with a secondary probe.

**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

## Counterstain:

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

## Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

## Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI210 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** HMW CK+p63

**Protocol Template (Description):** Ms HRP Template 1

**Dewaxing (DS Buffer Option):** DS2-50

**Antigen Retrieval (AR Option):** AR2, low pH; 101°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** HMW CK+p63, 30 min., 25°C

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## 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.

## 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 ( $\text{NaN}_3$ ) 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) (6)
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. (7)
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. 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.
2. 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.
3. Wang Y, *et al*. Cell differentiation lineage in the prostate. *Differentiation*. 2001 Oct;68(4-5):270-9.
4. 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.
5. 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.
6. 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."
7. 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.