p63

Concentrated and Prediluted Monoclonal Antibody
901-163-031519

BIOCARE MEDICAL

Catalog Number:	CM 163 A, B, C	PM 163 AA, H	IP 163 G10	OAI 163 T60	VP 163 G, G25	VLTM 163 G20
Description:	0.1, 0.5, 1.0 mL, conc.	6.0, 25 mL, RTU	10 mL, RTU	60 tests, RTU	6.0, 25 mL, RTU	20 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Van Gogh Yellow	N/A	N/A	N/A	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
p63 [4A4] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of p63 protein 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:
p63, a homolog of the tumor suppressor p53, has been identified in basal cells in the epithelial layers of a variety of tissues, including epidermis, cervix, urothelium, breast and prostate (1). p63 was detected in nuclei of the basal epithelium in normal prostate glands; however, it was not expressed in malignant tumors of the prostate (2). As a result, p63 has been reported as a useful marker for differentiating benign from malignant lesions in the prostate, particularly when used in combination with markers of high molecular weight cytokeratins and the prostate-specific marker AMACR (P504S) (3-4).
p63 has also been shown to be a sensitive marker for lung squamous cell carcinomas (SqCC), with reported sensitivities of 80-100% (5-8). Specificity for lung SqCC, vs. lung adenocarcinoma (LADC), has been reported to be approximately 70-90%, as positive staining with p63 has been typically observed in 10-30% of LADC cases (5-8).
In breast tissue, p63 has been identified in myoepithelial cells of normal ducts (9). Reports have described the utility of p63 in a panel of IHC markers for the assessment of breast lesions, due to the differential expression of the luminal vs. basal and myoepithelial markers (9-11).

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, mouse and rat
Clone: 4A4
Isotype: IgG2a/kappa
Protein Concentration: Call for lot specific Ig concentration
Epitope/Antigen: p63
Cellular Localization: Nuclear
Positive Tissue Control: Normal prostate

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after

Storage and Stability Cont'd:
expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTM163 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-Hi pH, 5X (use at 1X).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block (Optional): Incubate for 10-20 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 Peroxidazed 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.
intelliPATH FLX Automated Slide Stainer:
IP163 is intended for use with the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.
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™ Automated Slide Staining System):
OAI163 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: p63
Protocol Template (Description): Ms HRP Template 1
Dewaxing (DS Option): DS2
Antigen Retrieval (AR Option): AR1, high pH; 101°C
Reagent Name, Time, Temp.: p63, 30 min., 25°C

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Protocol Recommendations (Ventana BenchMark XT / ULTRA):
VP163 is intended for use with the BenchMark XT / ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using **ultraView on XT / ULTRA:**

Template/Detection: ultraView DAB
Pretreatment Protocol: ULTRA CC1 Standard
Primary Antibody: 32 minutes, 37°C
ultraBlock (V-Blocker BRI4001): Incubate for 4 minutes (with appropriate Option # registered by user)
V-Blocker is recommended to be applied prior to any detection system.

- Using **OptiView on ULTRA:**

Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 64 minutes
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 32 minutes, 36°C

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 (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) (12)
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 in contact with sensitive areas, wash with copious amounts of water. (13)
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. 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.
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.

References Cont'd:

3. Paner GP, Luthringer DJ, Amin MB. Best practice in diagnostic immunohistochemistry: prostate carcinoma and its mimics in needle core biopsies. Arch Pathol Lab Med. 2008 Sep; 132(9):1388-96.
4. Humphrey PA. Diagnosis of adenocarcinoma in prostate needle biopsy tissue. J Clin Pathol. 2007 Jan; 60(1):35-42.
5. Mukhopadhyay S, Katzenstein AL. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: Utility of an immunohistochemical panel containing TTF-1, napsin A, p63, and CK5/6. Am J Surg Pathol. 2011 Jan; 35(1):15-25.
6. Tacha D, *et al.* A six antibody panel for the classification of lung adenocarcinoma versus squamous cell carcinoma. Appl immunohistochem Mol Morphol. 2012 May; 20 (3):201-7.
7. Terry J, *et al.* Optimal immunohistochemical markers for distinguishing lung adenocarcinomas from squamous cell carcinomas in small tumor samples. Am J Surg Pathol. 2010 Dec; 34(12):1805-11.
8. Pu RT, Pang Y, Michael CW. Utility of WT-1, p63, MOC31, mesothelin, and cytokeratin (K903 and CK5/6) immunostains in differentiating adenocarcinoma, squamous cell carcinoma, and malignant mesothelioma in effusions. Diagn Cytopathol. 2008 Jan; 36(1):20-5.
9. Lerwill MF. Current practical applications of diagnostic immunohistochemistry in breast pathology. Am J Surg Pathol. 2004 Aug; 28(8):1076-91.
10. Hicks DG. Immunohistochemistry in the diagnostic evaluation of breast lesions. Appl Immunohistochem Mol Morph. 2011 Dec; 19(6):501-5.
11. Yeh IT, Mies C. Application of immunohistochemistry to breast lesions. Arch Pathol Lab Med. 2008 Mar; 132(3):349-58.
12. 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."
13. 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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