

## p63, 2X (Lung)

Prediluted Monoclonal Antibody  
Control Number: 901-3070-090917

**Catalog Number:** API 3070 AA  
**Description:** 6.0 ml, prediluted  
**Dilution:** Ready-to-use  
**Diluent:** N/A

**Intended Use:**

For In Vitro Diagnostic Use

p63, 2X (Lung) [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 lung 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 has been shown to be a sensitive marker for lung squamous cell carcinomas (SqCC), with reported sensitivities of 80-100% (1-6). Specificity for lung SqCC, versus 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 (1-6).

Cocktails of p63 with complementary markers for lung SqCC have also proven useful. A cocktail of p63 + TRIM29 demonstrated a 94.7% sensitivity for lung SqCC and 100% specificity versus LADC, in cases where Napsin A and TTF-1 were both negative (2-7). Similarly, the combination of p63 + CK5 identified 87% of cases of lung SqCC, with 94% specificity (8).

**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, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; mouse and rat

**Clone:** 4A4

**Isotype:** IgG2a/kappa

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.

**Epitope/Antigen:** p63

**Cellular Localization:** Nuclear

**Positive Tissue Control:** Lung squamous cell carcinoma

**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 Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.

**Protein Block:** Incubate for 10 minutes at RT with Biocare's Background Punisher.

**Primary Antibody:** Incubate for 15 minutes at RT.

**Probe:** N/A

**Polymer:** Incubate for 30 minutes at RT with a secondary-conjugated polymer.

**Chromogen:**

Incubate for 5 minutes at RT with Biocare's DAB.

**Counterstain:**

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

**Technical Note:**

1. This antibody has been standardized with Biocare's MACH 2 DS 2 detection system.
2. Use TBS buffer for washing steps.
3. If this reagent is used in combination with other primary antibodies, the antibody incubation time may need to be extended up to 30 minutes, depending upon the particular protocol of the individual investigator.

**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](http://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<sub>3</sub>) 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) (9)
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. (10)
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 MSDS is available upon request and is located at <http://biocare.net/support/msds/>.

**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.

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**References:**

1. 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.
2. 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.
3. Kargi A, Gurel D, Tuna B. The diagnostic value of TTF-1, CK 5/6, and p63 immunostaining in classification of lung carcinomas. *Appl Immunohistochem Mol Morphol.* 2007 Dec; 15(4):415-20.
4. Khayyata S, *et al.* Value of P63 and CK5/6 in distinguishing squamous cell carcinoma from adenocarcinoma in lung fine-needle aspiration specimens. *Diagn Cytopathol.* 2009 Mar; 37:178-83.
5. 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.
6. 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.
7. Tacha D, Yu C, Haas T. TTF-1, Napsin A, p63, TRIM29, Desmoglein-3 and CK5: An Evaluation of Sensitivity and Specificity and Correlation of Tumor Grade for Lung Squamous Cell Carcinoma vs. Lung Adenocarcinoma. *Mod Pathol.* 2011 Feb; 24 (Supplement 1s):425A.
8. Tacha D, Zhou D, Henshall-Powell RL. Distinguishing Adenocarcinoma from Squamous Cell Carcinoma in Lung Using Double Stains p63+ CK5 and TTF-1 + Napsin A. *Mod Pathol.* 2010 Feb; 23 (Supplement 1s):222A.
9. 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."
10. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory workers from occupationally Acquired Infections; Approved guideline-Third Edition CLSI document M29-A3 Wayne, PA 2005.