

p63 + CK5

Prediluted Multiplex Antibody Reagent

Control Number: 902-391DS-051515

Catalog Number: APR 391 DS AA

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For Research Use Only. Not for use in diagnostic procedures.

Summary and Explanation:

The p63 + CK5 Multiplex IHC stain has been especially designed for squamous cell carcinomas, particularly those derived in lung cancer. In-house studies have shown greater than 80% of squamous cell carcinoma of the lung were positive and other studies have shown that the combination of p63 and CK5 was useful for differentiating adenocarcinoma from squamous cell carcinoma with 100% specificity and 82% sensitivity, 89% specificity and 79% sensitivity, respectively. Studies have also shown that TTF-1 and Napsin A are highly specific and sensitive for lung adenocarcinomas. A critical assessment is essential for correct diagnosis because patients with squamous carcinoma (SqCC) cannot receive Avastin therapy due to a 30% mortality rate as a result of fatal hemoptysis (hemorrhaging). Therefore when used in a panel with TTF-1 + Napsin A, this unique antibody cocktail of p63 + CK5 should prove useful for immunohistochemical analysis of poorly differentiated lung adenocarcinomas vs. squamous cell carcinomas in formalin-fixed paraffin-embedded tissues.

Principle of Procedure:

This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Reagent Provided:

p63 + CK5 is provided as a prediluted antibody cocktail of anti-p63 and anti-CK5 antibodies, in buffer with carrier protein and preservative.

Antibody	anti-p63	anti-CK5
Clone	4A4	EP42*
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG2a/kappa	IgG
Epitope/ Antigen	p63	C-terminal region of CK5
Cellular Localization	Nuclear	Cell Surface/Cytoplasmic
Staining	Brown (DAB)	Red (Warp Red)

*Previously known as EP1601Y

Storage and Stability:

Store at 2°C to 8°C. Do not use reagent after the expiration date printed on the vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues).

Species Reactivity: Human, others not tested

Positive Tissue Control: Lung squamous cell carcinoma

Staining Protocol Recommendations:

Deparaffinization and rehydration: Perform deparaffinization of tissues with xylenes or xylene substitute, followed by rehydration through graded alcohols.

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 30-60 minutes at RT.

Double Stain Detection: Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2.

Chromogen (1): Incubate for 5 minutes at RT with Biocare's Betazoid DAB.

Chromogen (2): Incubate for 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.

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

Technical Notes:

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

Limitations:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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 (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) (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 in 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>.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.



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References:

1. Downey P, *et al.* If it's not CK5/6 positive, TTF-1 negative it's not a squamous cell carcinoma of lung. *APMIS*. 2008 Jun;116(6):526-9.
2. Kargi A, *et al.* 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.
3. 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(3):178-83.
4. Rossi G, *et al.* Morphology and a limited number of immunohistochemical markers may efficiently subtype non-small-cell lung cancer. *J Clin Oncol*. 2009 Oct;27(28):e141-2.
5. Bishop JA, Sharma R, Illei PB. Napsin A and TTF-1 expression in carcinomas of the lung, breast, pancreas, colon, kidney, thyroid, and malignant mesothelioma. *Human Pathol*. 2010 Jan;41(1):20-5.
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.

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