p63 + CK5

Prediluted Multiplex Antibody Reagent
901-391DS-061919

BIOCARE MEDICAL

Catalog Number:	**PM 391DS AA**	**VLTMR 391 G20**
Description:	6.0 mL, RTU	20 mL, RTU
Dilution:	Ready-to-use	Ready-to-use
Diluent:	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
p63 + CK5 is intended for laboratory use in the qualitative identification of p63 and cytokeratin 5 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:
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 (2). Studies have also shown that TTF-1 and Napsin A are highly specific and sensitive for lung adenocarcinomas (5). 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) (3). 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 (1-4).

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 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

*Previously known as EP1601Y

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.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human; others not tested
Positive Tissue Control: Lung squamous cell carcinoma

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTMR391 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: Incubate for 10 minutes with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Double Stain Detection: Incubate for 30 minutes using Val Plex 2.
Chromogen (1): Incubate for 5 minutes with Val DAB.
Chromogen (2): Incubate for 15 minutes with Val Fast Red.
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 Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.
Protein Block: Incubate for 10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30-60 minutes at RT.
Double Stain Detection: Incubate for 30 minutes at RT using MACH 2 Double Stain 2.
Chromogen (1): Incubate for 5 minutes at RT with Betazoid DAB.
Chromogen (2): Incubate for 5-7 minutes at RT with 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, for intelliPATH FLX and manual use, has been standardized with MACH 2 Double Stain 2. Use TBS buffer for washing steps.

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

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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) (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. 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, 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.
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.

Produced using Abcam's RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.

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