p63 + TRIM29

Prediluted Multiplex Antibody Reagent
901-427DS-061919

BIOCARE MEDICAL

Catalog Number:	**PPM 427DS AA**	**VLTMR 427 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 + TRIM29 is intended for laboratory use in the qualitative identification of p63 and TRIM29 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 patients clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
Tumor protein p63, also known as transformation-related protein 63 is a protein that in humans is encoded by the TP63 gene. Many studies have shown that p63 is a sensitive and fairly specific marker for squamous cell carcinoma and may be used in distinguishing poorly differentiated squamous cell carcinomas from adenocarcinomas. p63 has been shown to mark approximately 5 to 10% of lung adenocarcinomas (1-3).
Tripartite motif-containing 29 (TRIM29) is a relatively new marker. A comprehensive study has shown that TRIM29 is a sensitive marker (92.6%) for lung squamous cell carcinoma (SqCC) and is a fairly specific marker staining only 7.0% of lung adenocarcinomas (1).
p63 is a nuclear stain that marks lung SqCC (DAB), and TRIM29 is a cytoplasmic/membrane stain that also marks lung SqCC (Red). In most cases, a co-expression of both antibodies will be observed in lung SqCC. Studies have also shown that when p63 and/or TRIM29 is expressed in lung SqCC, a 94.7% sensitivity and 100% specificity was achieved, if Napsin A and TTF-1 were both negative in the same case (2). Therefore, the antibody cocktail of p63 + TRIM29 may provide an excellent screening tool for discriminating lung SqCC vs. lung adenocarcinoma.

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 + TRIM29 is provided as a prediluted antibody cocktail of anti-p63 and anti-TRIM29 antibodies, in buffer with carrier protein and preservative.

Antibody	anti-p63	anti-TRIM29
Clone	4A4	N/A
Source	Mouse monoclonal	Rabbit polyclonal
Isotype	IgG2a/kappa	IgG
Epitope/ Antigen	p63	TRIM29
Cellular Localization	Nuclear	Cytoplasmic & membrane
Staining	Brown (DAB)	Red

Storage and Stability:
Store at 2ºC to 8ºC. 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.

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):
VLTMR427 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 45 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 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 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.

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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) (5)
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. (6)
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 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. Modern Pathology; Volume 24, Supplement 1, Feb 2011, Abstract 1808, page 425A.
2. Tacha D, Zhou D, Henshall-Powell RL[1]. Distinguishing Adenocarcinoma from Squamous Cell Carcinoma in Lung Using Double Stains p63+ CK5 and TTF-1 + Napsin A. Modern Pathology; Pathology Volume 23, Supplement 1, Feb 2010; Abstract 1852, page 415A-416A.
3. 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.
4. Ring BZ, *et al.* A novel five-antibody immunohistochemical test for subclassification of lung carcinoma. Mod Pathol. 2009 Aug; 22(8):1032-43.
5. 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."
6. 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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