p63 + TRIM29

Prediluted Multiplex Antibody Reagent

Control Number: 902-427DS-091117

Catalog Number: **APR 427DS 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:

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 (Warp 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 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 + 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	Rabbit IgG
Epitope/Antigen	p63	TRIM29
Cellular Localization	Nuclear	Cytoplasmic & membrane
Staining	Brown (DAB)	Red (Warp 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

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 Peroxidazed 1 (BRR968).

Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker (BRR2004B or BRR2004C). Refer to the Diva Decloaker product data sheet for specific instructions.

Protein Block: Incubate for 10 minutes at RT with Biocare's Background Punisher (BRR974).

Primary Antibody: Incubate for 30 minutes at RT.

Double Stain Detection: Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2 (BRR525A).

Chromogen (1): Incubate for 5 minutes at RT with Biocare's Betazoid DAB (BRR2004A).

Chromogen (2): Incubate for 5-7 minutes at RT with Biocare's Warp Red (BRR806A). Rinse in deionized water.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution (BRRHTBLU) 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 (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 in 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 MSDS is available upon request and is located at http://biocare.net/support/msds/.

Technical Support:

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

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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. Mod Pathol. 2011 Feb;24 (Supplement 15):425A

2. 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. 2011 Feb;23 (Supplement 15):414A

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-Third Edition CLSI document M29-A3 Wayne, PA 2005.

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