Desmoglein 3 + Napsin A

Prediluted Multiplex Antibody Reagent

Control Number: 902-428DS-091117

Catalog Number: **APR 428DS 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:

Desmoglein 3 (DSG3) is a calcium-binding transmembrane glycoprotein component of desmosomes in vertebrate epithelial cells. Studies have shown DSG3 to have 83-95% sensitivity and 100% specificity in detecting squamous cell carcinoma (SqCC) vs. lung adenocarcinoma (1). DSG3 is associated with shorter survival for all lung cancer patients regardless of the histologic subtype (5-year survival of 20.9% vs. 49.5%. Patients with atypical carcinoid tumors, lacking Desmoglein 3 expression showed a 5-year survival of 0% compared with 36.8% for DSG3 positive cases.

Napsin A is expressed in type II pneumocytes of normal lung and in adenocarcinomas of the lung and kidney. Studies have shown that Napsin A is more sensitive (80-87%) and more specific marker than TTF-1. Napsin A is 100% specific for lung adenocarcinoma vs. 100% negative in lung SqCC (2). Studies have shown Napsin A used in combination with TTF-1 provides 93% sensitivity and 100% specificity for lung adenocarcinoma, if CK5 and Desmoglein 3 are both negative in the same section (2).

DSG3 is a cell membrane stain that marks lung SqCC (DAB). Napsin A is a cytoplasmic/granular stain that marks lung adenocarcinomas (Warp Red). In the vast majority of lung cancers tested, only a single antibody stain will be observed. Studies have shown co-expression of both antibodies may be observed in adenosquamous cell carcinomas, or in some cases residual normal lung will stain with Napsin A (1-3). Studies have shown this antibody cocktail is extremely accurate and is 100% specific. In grades 1-2, Desmoglein 3 + Napsin A provide staining sensitivity in the mid 90% range; thus the antibody cocktail of Desmoglein 3 + Napsin A is a first-line screener for discriminating between lung adenocarcinoma vs. lung SqCC (1).

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:

Desmoglein3 + Napsin A is provided as a prediluted antibody cocktail of anti-Desmoglein 3 and anti-Napsin A antibodies, in buffer with carrier protein and preservative.

Antibody	anti-Desmoglein 3	anti-Napsin A
Clone	BC11	N/A
Source	Mouse monoclonal	Rabbit polyclonal
Isotype	IgG1	N/A
Epitope/Antigen	Desmoglein 3	Napsin A
Cellular Localization	Membrane	Cytoplasmic - granular
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 and lung adenocarcinoma

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 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 is 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:

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

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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. 2010 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. Savci-Heijink CD, *et al*. The role of desmoglein-3 in the diagnosis of squamous cell carcinoma of the lung. Am J Pathol. 2009 May; 174(5):1629-37.

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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