Desmoglein 3 + p40 (M) + Napsin A (RM)

Prediluted Multiplex Antibody Reagent

Control Number: 901-3132DS-090817

Catalog Number: **API 3132DS AA**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use

Desmoglein 3 + p40 (M) + Napsin A (RM) is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of Desmoglein 3, p40 and Napsin A 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:

Desmoglein 3 (DSG3) is a calcium-binding transmembrane glycoprotein component of desmosomes in vertebrate epithelial cells. Currently, three desmoglein subfamily members have been identified and all are members of the cadherin cell adhesion molecule superfamily (1,2). Desmogleins exhibit membranous expression and connect with cytokeratins through desmoplakins and plakoglobin. DSG3 is particularly important in the cellular adhesion of squamous epithelium, and as a result, it is often highly expressed in various squamous cell carcinomas (SqCC) (3). In lung SqCC specifically, DSG3 has demonstrated a sensitivity of 85-100%, and an ability to discriminate lung adenocarcinoma (ADC) with a specificity of 98-100% (3-6).

The mouse monoclonal antibody p40 [BC28] recognizes an epitope unique to the p40 protein and may have applications in cases where p63 has traditionally been used. To date, p63 [4A4] has been a frequently used marker for lung SqCC, as well as for bladder, breast, prostate, and head and neck cancers. p63 [4A4] recognizes both the p63 and p40 proteins (7). As a result, p63 [4A4] has proven to be a sensitive marker for lung SqCC; however, it suffers from specificity limitations due to reactivity in a subset of lung ADC. In contrast, p40 is selectively expressed in lung SqCC, offering an opportunity for improved specificity (5-8). p40 (M) [BC28] recognizes an epitope unique to p40, which may result in diminished reactivity in lung ADC and increased specificity (8).

The combination of both membrane and nuclear staining of DSG3 and p40, respectively, may increase overall sensitivity for lung SqCC and, in some cases, may aid the pathologist with difficult specimens (4,5).

Napsin A is a pepsin-like aspartic proteinase. It is expressed in most lung adenocarcinomas and some renal cell carcinomas, as well as in type II pneumocytes (4-6). Studies have shown that Napsin A is a more sensitive and specific marker than TTF-1 and is extremely specific for lung ADC versus lung SqCC (4-6).
U.S. Patent 9,428,576 and patents pending.

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:

Desmoglein 3 + p40 (M) + Napsin A (RM) is provided as a prediluted antibody cocktail of anti-Desmoglein 3, anti-p40 (M) and anti-Napsin A (RM) antibodies, in buffer with carrier protein and preservative.

Antibody	anti-Desmoglein	anti-p40	anti-Napsin A
Clone	BC11	BC28	BC15
Source	Mouse monoclonal	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1	IgG1	IgG
Epitope/ Antigen	Desmoglein 3	amino acids 5-17	Napsin A
Cellular Localization	Membrane	Nuclear	Cytoplasmic (granular)
Staining	Brown (DAB)	Brown (DAB)	Red (Warp Red)

Storage and Stability:

Store at 2ºC to 8ºC. 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 and lung adenocarcinoma

Protocol Recommendations:

Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 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 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. 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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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) (9)
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. (10)
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. Buxton RS, Magee AI. Structure and interactions of desmosomal and other cadherins. Semin Cell Biol. 1992 Jun; 3(3):157-67.
2. North AJ, *et al.* Molecular map of the desmosomal plaque. J Cell Sci. 1999 Dec; 112 (Pt 23):4325-36.
3. 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.
4. Tacha D, *et al.* A six antibody panel for the classification of lung adenocarcinoma versus squamous cell carcinoma. Appl Immunohistochem Mol Morphol. 2012 May; 20 (3):201-7.
5. Brown AF, *et al.* Tissue-preserving antibody cocktails to differentiate primary squamous cell carcinoma, adenocarcinoma, and small cell carcinoma of lung. Arch Pathol Lab Med. 2013 Sep; 137(9):1274-81.
6. Agackiran Y, *et al.* Desmoglein-3 and Napsin A double stain, a useful immunohistochemical marker for differentiation of lung squamous cell carcinoma and adenocarcinoma from other subtypes. Appl Immunohistochem Mol Morphol. 2012 Jul; 20(4):350-5.
7. Bishop JA, *et al*. p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol. 2012 Mar; 25(3):405-15.
8. Tacha D, *et al*. An immunohistochemical analysis of a newly developed, mouse monoclonal p40 (BC28) antibody in lung, bladder, skin, breast, prostate, and head and neck cancers. Arch Pathol Lab Med. 2014 Oct; 138(10):1358-64.
9. 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."
10. 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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