Desmoglein 3 + Napsin A Cocktail
Prediluted Multiplex Cocktail (4-Step)
Control Number: 901-428DS-021611

Catalog Number: PPM 428DS AA

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use

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. 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%, P < .001). Patients with atypical carcinoid tumors, lacking Desmoglein 3 expression showed a 5-year survival of 0% compared with 36.8% for DSG3 positive cases (P < .001).

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. 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.

DSG3 is a cell membrane stain that marks lung SqCC (DAB). Napsin A is a cytoplasmic/granular stain that marks lung adenocarcinomas (Fast Red). In the vast majority of lung cancers tested, only a single antibody stain will be observed. Co-expression of both antibodies may be observed in adenosquamous cell carcinomas, or in some cases residual normal lung will stain with Napsin A. Desmoglein 3 + Napsin A are very sensitive and specific markers for discriminating between lung SqCC and lung adenocarcinoma. 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.

Principle of Multiplex Staining:
A Multiplex IHC stain can be accomplished in four major steps. The initial step consists of an antibody cocktail with at least one mouse and one rabbit antibody. This cocktail is applied to the tissue and will bind with two or more target antigens. A multiplex detection cocktail of horseradish peroxidase (HRP) and alkaline phosphatase (AP) conjugated secondary antibodies is applied. The third step consists of the addition of DAB-Substrate that binds to the HRP and produces a brown chromogenic reaction product. The fourth step consists of a Fast Red-Substrate that binds to the AP and produces a red chromogenic reaction product.

Source: Mouse monoclonal and Rabbit polyclonal

Species Reactivity: Human, others not tested

Clone: BC11 + N/A

Isotype: IgG1 + N/A

Epitope/Antigen: Desmoglein 3 + Napsin A

Cellular Localization:
Desmoglein 3 (Membrane): Brown
Napsin A (Cytoplasmic - granular): Red

Positive Control:
Lung squamous cell carcinoma and lung adenocarcinoma

Normal Tissue:
Skin or tonsil (DSG3); lung (Napsin A)

Abnormal Tissue:
Lung squamous cell carcinoma and lung adenocarcinoma

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative.

Protocol Recommendations

Peroxide Block:
Block for 5 minutes with Biocare's Peroxidazed 1.

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:
Heat Retrieval Method:
Retrieve sections using Biocare’s Decloaking Chamber at 125°C for 30 seconds. Allow solution to cool for 10 minutes then wash in distilled water

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 Note:
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.

Performance Characteristics:
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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:
Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about tissue controls.

Precautions:
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 (NaN3) 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)
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.
Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

Further information can be found at www.biocare.net for Biocare's Product Guide (MM4-A) Vol.19, No.26.
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Prediluted Multiplex Cocktail (4-Step)
Control Number: 901-428DS-021611

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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

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.

Limitations and Warranty:
There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

References:
2. Tacha D, Zhou D, Henshall-Powell RL¹. 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 222A.
p63 + TRIM29 Cocktail (SqCC)
Prediluted Multiplex Cocktail (4-Step)
Control Number: 901-427DS-021611

Catalog Number: PPM 427DS AA
Description: 6.0 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use

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 (90%) 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.

Tripartite motif-containing 29 (TRIM29) is a relatively new marker. A comprehensive study has shown that TRIM29 is a sensitive marker (93.7%) for lung squamous cell carcinoma (SqCC) and is a fairly specific marker staining only 6.1% of lung adenocarcinomas.

p63 is a nuclear stain that marks lung SqCC (DAB), and TRIM29 is a cytoplasmic/membrane stain that also marks lung SqCC (Fast 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 95.4% sensitivity and 100% specificity was achieved, if Napsin A and TTF-1 were both negative in the same case. Therefore, the antibody cocktail of p63 + TRIM29 is an excellent screener for discriminating lung SqCC vs. lung adenocarcinoma.

Principle of Multiplex Staining:
A Multiplex IHC stain can be accomplished in four major steps. The initial step consists of an antibody cocktail with at least one mouse and one rabbit antibody. This cocktail is applied to the tissue and will bind with two or more target antigens. A multiplex detection cocktail of horseradish peroxidase (HRP) and alkaline phosphatase (AP) conjugated secondary antibodies is applied. The third step consists of the addition of DAB-Substrate that binds to the HRP and produces a brown chromogenic reaction product. The fourth step consists of a Fast Red-Substrate that binds to the AP and produces a red chromogenic reaction product.

Source: Mouse monoclonal and Rabbit polyclonal
Species Reactivity: Human, others not tested
Clone: BC4A4 + N/A
Isotype: IgG2a/kappa + Rabbit IgG
Epitope/Antigen: p63 + TRIM29

Cellular Localization:
p63 (Nuclear): Brown
TRIM29 (Cytoplasmic & membrane): Red

Positive Control: Lung squamous cell carcinoma
Normal Tissue: Prostate, bladder (p63); prostate, placenta (TRIM29)
Abnormal Tissue: Lung squamous cell carcinoma

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative.

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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations
Peroxide Block:
Block for 5 minutes with Biocare's Peroxidazet 1.

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:
Heat Retrieval Method:
Retrieval sections using Biocare's Decloaking Chamber at 125ºC for 30 seconds. Allow solution to cool for 10 minutes then wash in distilled water.

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 Note:
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.

Performance Characteristics:
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Quality Control:
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Pretreatment Solution (recommended): Diva

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