TTF-1 + Napsin A

Prediluted Multiplex Antibody Reagent

Control Number: 902-394DS-082217

Catalog Number: **APR 394 DS 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:

TTF-1 has been the premier marker for lung adenocarcinoma. A new and promising marker, Napsin A, is expressed in type II pneumocytes and in adenocarcinomas of the lung (2). Studies have shown Napsin A to be more sensitive and specific than TTF-1 in lung adenocarcinomas and virtually negative in all squamous carcinomas. In other studies comparing TTF-1 and SP-A, Napsin A stained more tumor cells and a higher percentage of lung adenocarcinomas than either of these antibodies (1-3). Other studies have shown that when TTF-1 and Napsin A are used in combination, a higher sensitivity and specificity is achieved (4). A critical assessment is essential for correct diagnosis as patients with squamous carcinoma (SqCC) cannot receive Avastin due to a 30% mortality rate as a result of fatal hemoptysis (hemorrhaging). Therefore, when used in a panel with p63 and CK5, this unique multiplex antibody reagent of TTF-1 and Napsin A may aid in the analysis of poorly differentiated lung adenocarcinomas vs. squamous cell carcinomas in formalin-fixed paraffin-embedded tissues.

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:

TTF-1 + Napsin A is provided as a prediluted antibody cocktail of anti-TTF-1 and anti-Napsin A antibodies, in buffer with carrier protein and preservative.

Antibody	anti-TTF-1	anti-Napsin A
Clone	8G7G3/1	N/A
Source	Mouse monoclonal	Rabbit polyclonal
Isotype	IgG1	Rabbit IgG
Epitope/Antigen	TTF-1	Napsin A
Cellular Localization	Nuclear	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 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:

1. Literature reports suggest that high pH antigen retrieval solutions should not be used when staining TTF-1. Therefore, antigen retrieval with Diva (pH 6.2) is strongly recommended.
2. 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.
3. A longer primary antibody incubation may be required to enhance staining.

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. Hirano T, Gong Y, Yoshida K, Kato Y, Yashima K, Maeda M, Nakagawa A,Fujioka K, Ohira T, Ikeda N, Ebihara Y, Auer G, Kato H. Usefulness of TA02 (Napsin A) to distinguish primary lung adenocarcinoma from metastatic lung adenocarcinoma. Lung Cancer. 2003 Aug; 41(2):155-62.
2. Ueno T, Linder S, Elmberger G. Aspartic proteinase napsin is a useful marker for diagnosis of primary lung adenocarcinoma. Br J Cancer. 2003 Apr 22; 88(8):1229-33.
3. Suzuki A, Shijubo N, Yamada G, Ichimiya S, Satoh M, Abe S, Sato N. Napsin A is useful to distinguish primary lung adenocarcinoma from adenocarcinomas of other organs. Pathol Res Pract. 2005; 201 (8-9):579-86.
4. Dejmek A, Naucler P, Smedjeback A, Kato H, Maeda M, Yashima K, Maeda J, Hirano T. Napsin A (TA02) is a useful alternative to thyroid transcription factor-1 (TTF-1) for the identification of pulmonary adenocarcinoma cells in pleural effusions. Diagn Cytopathol. 2007 Aug; 35(8):493-7.
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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