

TTF-1 + Napsin A (RM)

Prediluted Multiplex Antibody Reagent

Control Number: 901-3078DS-082217

Catalog Number: API 3078 DS AA

Description: 6.0 ml, Prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use

TTF-1 + Napsin A is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of TTF-1 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:

TTF-1 is a member of the NKX2 family of homeodomain transcription factors found in epithelial cells of the thyroid gland and lung. TTF-1 has been shown to stain approximately 65-70% of cases of lung adenocarcinomas (ADC), with a small number of cases of lung squamous cell carcinoma (SCC) also positive for TTF-1 (1-4).

Napsin A is an aspartic proteinase expressed in type II pneumocytes and in adenocarcinomas of the lung (1). Napsin A has been found to be a sensitive and specific marker of lung ADC. Napsin A has been shown to positively stain 85-90% of cases of lung ADC, while negative staining has been observed in nearly all cases of lung SCC (1-6).

The antibody cocktail of TTF-1 + Napsin A has demonstrated a sensitivity of 91% (105/115) for lung ADC and 95% specificity (5/95) versus lung SCC (2). In cases where TTF-1 and Napsin A are both positive, specificity versus lung squamous cell carcinoma has been shown to be 100% (2).

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 (RM) 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	BC15
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1	IgG
Epitope/ Antigen	TTF-1	Napsin A
Cellular Localization	Nuclear	Cytoplasmic (granular)
Staining	Brown (DAB)	Red (Warp Red)

Storage and Stability:

Store at 2°C to 8°C. 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 Control: Lung adenocarcinoma

Protocol Recommendations:

Peroxide Block: Block for 5 minutes with Biocare's Peroxidized 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 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.

Chromogen (2): Incubate for 5 minutes at RT with Biocare's Betazoid DAB.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

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

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 (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) (7)
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. (8)

TTF-1 + Napsin A (RM)

Prediluted Multiplex Antibody Reagent

Control Number: 901-3078DS-082217

Precautions Cont'd:

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/>.

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. Ueno T, Linder S, Stetinger G. Aspartic proteinase napsin is a useful marker for diagnosis of primary lung adenocarcinoma. *Br J Cancer*. 2003 Apr 22; 88(8):1229-33.
2. Tacha D, *et al.* A 6-antibody panel for the classification of lung adenocarcinoma versus squamous cell carcinoma. *Appl Immunohistochem Mol Morphol*. 2012 May; 20(3):201-7.
3. Mukhopadhyay S, Katzenstein AL. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: Utility of an immunohistochemical panel containing TTF-1, napsin A, p63, and CK5/6. *Am J Surg Pathol*. 2011 Jan; 35(1):15-25.
4. Turner BM, *et al.* Napsin A, a new marker for lung adenocarcinoma, is complementary and more sensitive and specific than thyroid transcription factor 1 in the differential diagnosis of primary pulmonary carcinoma: evaluation of 1674 cases by tissue microarray. *Arch Pathol Lab Med*. 2012 Feb; 136(2):163-71.
5. Hirano T, *et al.* Usefulness of TA02 (Napsin A) to distinguish primary lung adenocarcinoma from metastatic lung adenocarcinoma. *Lung Cancer*. 2003 Aug; 41(2):155-62.
6. Suzuki A, *et al.* Napsin A is useful to distinguish primary lung adenocarcinoma from adenocarcinomas of other organs. *Pathol Res Pract*. 2005; 201(8-9):579-86.
7. 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."
8. 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.