Bio-Optica
Improving Pathology

BIOCARE MEDICAL

ISO 9001&13485 CERTIFIED

# TTF-1 + Napsin A

## Prediluted Multiplex Antibody Reagent

Control Number: 901-394DS-100314

| | | |
|---|---|---|
| **Catalog Number:** | **PPM 394 DS AA** | **IPI 394 DS G10** |
| **Description:** | 6.0 ml, prediluted | 10 ml, prediluted |
| **Dilution:** | Ready-to-use | Ready-to-use |
| **Diluent:** | N/A | N/A |

**Intended Use:**

For In Vitro Diagnostic Use

TTF-1 + Napsin A is a cocktail of mouse monoclonal and rabbit polyclonal 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 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 | 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

**Protocol Recommendations (intelliPATH and manual use):**

**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 or intelliPATH Multiplex Secondary Reagent 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:**

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 optimized for use with Biocare's MACH 2 Double Stain 2 and intelliPATH Multiplex Secondary Reagent 2. Other Biocare polymer detection kits may be used; however, users must validate incubation times and protocols for their specific application. Use TBS for washing steps.
3. A longer primary antibody incubation may be required to enhance staining.

**intelliPATH™ Automated Slide Stainer:**

IPI394DS is intended for use on the intelliPATH™ Automated Slide Stainer. Refer to the intelliPATH Automated Slide Stainer manual for specific instructions on its use. When using the intelliPATH, peroxide block with intelliPATH Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

**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 test.

**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) (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 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. 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.
2. Ueno T, Linder S, Steterger 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, *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.
4. Dejmek A, *et al.* 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-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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