

TTF-1 + p40 (cRM)

Prediluted Multiplex Antibody Reagent

Control Number: 901-3141DS-090917

Catalog Number: API 3141 DS AA

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use

TTF-1 + p40 (cRM) is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of TTF-1 and p40 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:

Thyroid transcription factor-1 (TTF-1) is a member of the NKX2 family of homeodomain transcription factors. Studies have shown that TTF-1 is expressed in epithelial cells of the thyroid gland and lung. TTF-1 has been shown to be a sensitive (62-80%) and specific marker (89-95%) in the majority of primary lung adenocarcinomas (ADC) (1-7). When combined in a cocktail with a rabbit polyclonal p40, TTF-1 was reported to demonstrate 77% sensitivity for lung ADC, and 100% specificity vs. lung squamous cell carcinoma (SCC) (7).

p40 has been shown to be selectively expressed in lung SCC, offering the potential for increased specificity compared to p63 (8-9). Mouse monoclonal p40 [BC28] recognizes an epitope unique to p40 and has been shown to be sensitive, staining 93% (99/107) of cases of lung SCC and specific, staining 0.8% (1/121) of cases of lung ADC (10).

Chimeric rabbit monoclonal rabbit p40 [BC28/cRM] was designed to replicate the sensitivity and specificity of mouse monoclonal p40 [BC28] as a rabbit antibody that would be suitable for a double-stain procedure. The variable regions of p40 [BC28], which define the binding sensitivity and specificity of the antibody, were cloned and fused with the constant regions of rabbit IgG to provide the chimeric rabbit monoclonal p40 [BC28/cRM]. In a side-by-side study on the same tissues, mouse monoclonal p40 [BC28] and chimeric rabbit monoclonal p40 [BC28/cRM] exhibited identical sensitivity (8/10) for lung SCC and specificity versus lung ADC (0/12). PATENT 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:

TTF-1 + p40 (cRM) is provided as a prediluted antibody cocktail of anti-TTF-1 and anti-p40 antibodies in buffer with carrier protein and preservative.

Antibody	anti-TTF-1	anti-p40
Clone	8G7G3/1	BC28/cRM
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1	IgG
Epitope/ Antigen	TTF-1	amino acids 5-17 of p40
Cellular Localization	Nuclear	Nuclear
Staining	Brown (DAB)	Red (Warp Red)

Storage and Stability:

Store at 2°C to 8°C. 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 adenocarcinoma (TTF-1); lung SCC (p40)

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 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

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) (12)

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

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Precautions Cont'd:

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. (13)

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. 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.
2. Tacha D, Yu C, Haas T. TTF-1, Napsin A, p63, TRIM29, Desmoglein-3 and CK5: An Evaluation of Sensitivity and Specificity and Correlation of Tumor Grade for Lung Adenocarcinoma versus Squamous Cell Carcinoma. *Mod Pathol.* 2011 Feb; 24 (Supplement 1s):425A.
3. Tacha D, Zhou D, Henshall-Powell RL. Distinguishing Adenocarcinoma from Squamous Cell Carcinoma in the Lung Using Multiplex IHC Stains: p63 + CK5 and TTF-1 + Napsin A. *Mod Pathol.* 2010 Feb; 23(Supplement 1s):414A.
4. 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.
5. Terry J, *et al.* Optimal immunohistochemical markers for distinguishing lung adenocarcinomas from squamous cell carcinomas in small tumor samples. *Am J Surg Pathol.* 2010 Dec; 34(12):1805-11.
6. Bishop JA, Sharma R, Illei PB. Napsin A and thyroid transcription factor-1 expression in carcinomas of the lung, breast, pancreas, colon, kidney, thyroid, and malignant mesothelioma. *Hum Pathol.* 2010 Jan; 41(1):20-5.
7. 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.
8. Bishop JA, *et al.* p40 is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol.* 2012 Mar; 25(3):405-15.
9. Hibi K, *et al.* AIS is an oncogene amplified in squamous cell carcinoma. *Proc Natl Acad Sci U S A.* 2000 May 9; 97(10):5462-7.
10. Tacha D, Bremer R, Haas T, Qi W. 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.
11. Pelosi G, *et al.* p40 and thyroid transcription factor-1 immunoreactivity on small biopsies or cellblocks for typing non-small cell lung cancer: a novel two-hit, sparing-material approach. *J Thorac Oncol.* 2012 Feb; 7(2):281-90.
12. 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."
13. 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.