

**TTF-1 + CK5**  
Prediluted Multiplex Antibody Reagent  
Control Number: 902-425DS-091117

**Catalog Number:** APR 425DS 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:**

Thyroid transcription factor-1 (TTF-1) is a member of the NKX2 family of homeodomain transcription factors. It is expressed in epithelial cells of the thyroid gland and lung. TTF-1 has been shown to be a sensitive (65-81%) and specific marker (94%) in the majority of primary lung adenocarcinomas. Studies have shown that TTF-1, used in combination with Napsin A, provided 91.3% sensitivity and 100% specificity for lung adenocarcinoma, if CK5 and Desmoglein 3 were both negative in the same case (2-3).

CK5 is a type II intermediate filament protein that is expressed in active basal layers of most stratified squamous epithelia. In a published study, rabbit monoclonal CK5 antibody was compared to mouse monoclonal CK5/6. CK5 was 79% sensitive and 100% specific for lung squamous cell carcinoma (SqCC) when compared to CK5/6 (75% sensitivity and 97% specificity) (3). CK6 mRNA has been detected in lung adenocarcinomas; thus CK5 alone, may be a more specific marker than CK5/6. Studies have shown that CK5, used in combination with Desmoglein 3, provided 93.7% sensitivity with 100% specificity for lung SqCC.

TTF-1 (lung adenocarcinoma) is stained with DAB chromogen, and CK5 rabbit monoclonal (lung SqCC) is stained with a Fast Red chromogen. In most lung cancers tested, only a single antibody stain will be observed. Co-expressions of both antibodies may be an indication of adenosquamous cell carcinomas. When used in combination with Desmoglein 3 and Napsin A, a 93% staining sensitivity and 100% specificity was achieved for lung adenocarcinoma, and a 93.7% sensitivity and 100% specificity was achieved for lung SqCC; therefore, the antibody cocktail of TTF-1 + CK5 is a first class screener for discriminating between lung adenocarcinoma (TTF-1) vs. lung SqCC (CK5) (4-6).

**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 + CK5 is provided as a prediluted antibody cocktail of anti-TTF-1 and anti-CK5 antibodies, in buffer with carrier protein and preservative.

Antibody	anti-TTF-1	anti-CK5
Clone	8G7G3/1	EP42*
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1	IgG
Epitope/ Antigen	TTF-1	C-terminal region of CK5
Cellular Localization	Nuclear	Cell Surface/Cytoplasmic
Staining	Brown (DAB)	Red (Warp Red)

\*Previously known as EP1601Y

**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

**Specific Tissue Control:** Lung adenocarcinoma (TTF-1); lung SqCC (CK5)

**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 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:**

- 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.
- This antibody has been standardized with Biocare's Double Stain 2. It can also be used on an automated staining system. Use TBS buffer for washing steps.

**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:**

- 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<sub>3</sub>) 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)
- 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)
- 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.
- Do not use reagent after the expiration date printed on the vial.
- The SDS is available upon request and is located at <http://biocare.net>.

**Technical Support:**

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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**References:**

1. 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.
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 Squamous Cell Carcinoma vs. Lung Adenocarcinoma. *Mod Pathol.* 2011 Feb;24 (Supplement 15):425A
3. Tacha D, Zhou D, Henshall-Powell RL. Distinguishing Adenocarcinoma from Squamous Cell Carcinoma in Lung Using Double Stains p63+ CK5 and TTF-1 + Napsin A. *Mod Pathol.* 2010 Feb;23 (Supplement 15):414A
4. 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.
5. Kargi A, Gurel D, Tuna B. The diagnostic value of TTF-1, CK 5/6, and p63 immunostaining in classification of lung carcinomas. *Appl Immunohistochem Mol Morphol.* 2007 Dec; 15(4):415-20.
6. Downey P, *et al.* If it's not CK5/6 positive, TTF-1 negative it's not a squamous cell carcinoma of lung. *APMIS.* 2008 Jun; 116(6):526-9.
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-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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