P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

TTF-1 & Cytokeratin 5 Multiplex Cocktail; Clones 8G7G3/1 & EP42
(Ready-To-Use)

Availability/Contents:

Item #	Volume
A00145-0002	2 ml
A00145-0007	7 ml
A00145-0025	25 ml

Description:

Species:	Mouse & Rabbit
Designation:	Mouse Monoclonal & Rabbit Monoclonal
Clones:	8G7G3/1 & EP42
Isotype:	IgG1 & IgG
Immunogen:	BALB/c mice were injected with recombinant rat TTF-1. Rabbits were injected with a synthetic peptide corresponding to residues near the C-Terminus of human Cytokeratin 5.
Format:	This antibody has been pretitered and in-house quality controlled to work on formalin-fixed paraffin-embedded as well as acetone fixed cryostat tissue sections. No further titration is required.
Specificity:	This antibody reacts with TTF-1 protein found in adenocarcinomas of the lung and tumors originating in the thyroid. TTF-1 positive cells are found in Type II pneumocytes and Clara cells in the lung. In the thyroid, follicular and parafollicular cells are positive. In lung cancers, Adenocarcinomas are usually positive, while Squamous Cell Carcinomas and Large Cell Carcinomas are rarely positive. In addition, Small-Cell Carcinomas (of any primary site) are usually positive. Cytokeratin 5 is expressed in many non-keratinizing stratified squamous epithelia including: basal epithelia, hair follicles, trachea, tongue mucosa, as well as basal cells in prostate glands and myoepithelial cells in mammary glands. Cytokeratin 5 protein is also found in most epithelial and bispasic mesotheliomas, large cell carcinoma and pulmonary squamous cell carcinomas. This Multiplex cocktail of TTF-1 and Cytokeratin 5 produces two-color high contrast staining for differentiating primary adenocarcinoma of the Lung from Metastatic Carcinomas of the breast and Malignant Mesothelioma.
Principle:	Multiplex IHC staining allows simultaneous testing of two or more analytes on a single tissue section utilizing different colors for each antigenic target.
Background:	Thyroid transcription factor (TTF-1) is a protein that regulates transcription of genes specific to thyroid, lung and diencephalon. It is also known as thyroid-specific enhancer binding protein and NKX-2. The protein plays a crucial role in normal lung function and morphogenesis. TTF-1 is expressed consistently throughout the life stages and uniformly in the terminal respiratory unit, which is comprised of peripheral airway cells and small-sized bronchioles.

Storage: 2° C 8° C

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The TTF-1 gene encodes a transcription termination factor that is localized to the nucleolus and plays a critical role in ribosomal gene transcription. The encoded protein mediates the termination of RNA polymerase I transcription by binding to Sal box terminator elements downstream of pre-rRNA coding regions. Alternatively spliced transcript variants encoding multiple isoforms have been observed for this gene.

TTF-1 is useful in differentiating primary Adenocarcinoma for the lung from Metastatic Carcinomas of the breast and Malignant Mesothelioma. The antibody can also be useful to differentiate Small-Cell Lung Carcinoma from lymphoid infiltrates.

Cytokeratin 5 is a type II Cytokeratin consisting of basic or neutral proteins which are arranged in pairs of heterotypic keratin chains and coexpressed during differentiation of simple and stratified epithelial tissues. The type II Cytokeratin genes are clustered in a region of chromosome 12q12-q13. Cytokeratin 5 is a 58kD protein that is closely related to Cytokeratin 6.

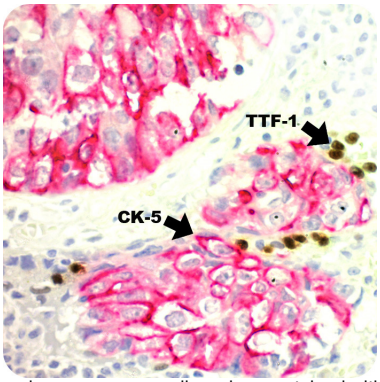
Cytokeratin 5 and calretinin have been useful markers suggestive of mesothelioma. Their expression is analyzed for the histological differential diagnosis with adenocarcinomas, especially when confronted with metastatic tumors of unknown origin. A combination of Cytokeratin 5, Cytokeratin 14, and p63 has been used as a sensitive and specific basal cell marker of basal-like phenotype of breast carcinoma and to differentiate normal and prostate cancer. Loss-of-function mutations in the Cytokeratin 5 gene affected family members and in six unrelated patients with Dowling-Degos disease (DDD) caused an autosomal dominant genodermatosis. This sugguests a crucial role for cytokeratins in the organization of cell adhesion, melanosome uptake, organelle transport and nuclear anchorage.

Species Reactivity:	Human. Others not tested.
Positive Control:	Adenocarcinoma of the Lung or Thyroid for TTF-1. Skin for normal tissue and Mesothelioma for abnormal tissue for Cytokeratin 5.
Cellular Localization:	TTF-1: Nuclear.
	Cytokeratin 5: Cytoplasmic / Cell Surface.
Titer/Working Dilution:	No further dilution is required.
Microbiological State:	This product is not sterile.

Uses/Limitations:

Not to be taken internally.
For Research Use Only.
This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy.
Do not use if reagent becomes cloudy.
Do not use past expiration date.
Non-Sterile.

Ordering Information and Current Pricing at www.scytek.com



Human lung squamous cell carcinoma stained with Ultra-Tek HRP using DAB Chromogen and UltraTek Alk-Phos using Permanent Red Chromogen.

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Procedure:

1. Deparaffinize and rehydrate tissue section.
2. **Tissue Section Pretreatment (Highly Recommended):** Staining of formalin fixed, paraffin embedded tissue sections is enhanced by pretreatment with Citrate Plus (ScyTek catalog# CPL500).
3. Wash 2 times in DI/Distilled water.
4. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in hydrogen peroxide for 10-15 minutes.
5. Wash 2 times in buffer.
6. Apply Super Block (Catalog# AAA) and incubate for 5 minutes at room temperature to block nonspecific background staining.
   Note: Do not exceed 10 minutes or there may be a reduction in desired stain.
7. Wash 3 times in buffer.
8. Apply primary antibody cocktail and incubate for 30 minutes in a humid environment.
9. Wash 3 times in buffer.
10. Apply UltraTek Anti-Mouse (Catalog# ABJ) and incubate for 10 minutes at room temperature.
11. Wash 3 times in buffer.
12. Apply UltraTek HRP (Catalog# ABL) and incubate for 10 minutes at room temperature.
13. Rinse 3 times in buffer.
14. Rinse 1 time in DI/Distilled water.
15. Apply mixed DAB Chromogen / DAB Substrate (Catalog# ACV) and incubate for 10-15 minutes, depending on the desired stain intensity.
    WARNING: DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.
16. Rinse 3 times in buffer.
17. Apply UltraTek Anti-Rabbit (Catalog# ABK) and incubate for 10 minutes at room temperature.
18. Rinse 3 times in buffer.
19. Rinse 1 time in DI/Distilled water.
20. Apply UltraTek Alk-Phos (Catalog# ABM) and incubate for 15 minutes at room temperature.
21. Rinse 3 times in buffer.
22. Rinse 1 time in DI/Distilled water.
23. Apply mixed Permanent Red Concentrate / Permanent Red Substrate (Catalog# PRD) and incubate for 10-15 minutes, depending on the desired stain intensity.
24. Rinse 2 times in DI/Distilled water.
25. Counterstain with Hematoxylin (Catalog# HMM) and coverslip using a permanent mounting media.

Precautions: Contains Sodium Azide as a preservative (0.09% w/v).
Do not pipette by mouth.
Avoid contact of reagents and specimens with skin and mucous membranes.
Avoid microbial contamination of reagents or increased nonspecific staining may occur.
This product contains no hazardous material at a reportable concentration according to U.S. 29 CFR 1910.1200, OSHA Hazardous Communication Standard and EC Directive 91/155/EC.

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Note: Cytokeratin 5 bearing EP Clone EP42 is Manufactured using Epitomics's RabMAb® technology under U.S. Patent Nos. 5,675,063 and 7,402,409.

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