

# Tissue-Tek Genie® DUO

## anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail

### Instructions for use

#### Intended use

For *in vitro* diagnostic use.

Tissue-Tek Genie® DUO anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail is an antibody cocktail designed to qualitatively detect cytokeratin 5/6 and TTF-1 proteins in formalin-fixed, paraffin embedded (FFPE) specimen sections by immunohistochemistry (IHC) staining on the Tissue-Tek Genie® Advanced Staining System. The clinical interpretation must be made in conjunction with histological examination, relevant clinical information, other diagnostic tests and proper controls by a qualified pathologist.

#### Limitations

This product has been optimized for use with the default protocol for this antibody on the Tissue-Tek Genie Advanced Staining System, using Tissue-Tek Genie® reagents and FFPE specimen sections. Staining quality may diminish when used with other systems and/or reagents.

#### Summary and principle

Cytokeratin 5 and 6 (CK5/6) are high molecular weight cytokeratins expressed in various basal cells and mesothelial cells. CK5/6 is expressed in basal cells of respiratory epithelium, skin, breast, prostate, esophagus, excretory ducts, and urinary track. CK5/6 is expressed in myoepithelial cells of breast and salivary gland acini as well as stratified mucosal epithelia of the esophagus and female genitor tract. CK5/6 is expressed in neoplasms derived from

stratified cutaneous and mucosal, including pulmonary squamous cell carcinoma. Some adenocarcinomas may also show focal staining of CK5/6, including lung, breast, and pancreas carcinomas. CK5/6 is generally not expressed in colorectal adenocarcinoma, stomach adenocarcinoma, renal cell carcinoma, thyroid carcinoma, neuroendocrine carcinoma, and germ cell tumors. CK5/6 is useful to help identify squamous cell carcinoma when used with a panel of other antibodies.

Thyroid Transcription Factor - 1 (TTF-1) is a transcription factor preferentially expressed in thyroid and lung. TTF-1 is generally not expressed in breast, colon, liver, and other tissues. TTF-1 is expressed in 89-93% of lung adenocarcinoma, 85-90% small cell lung carcinoma, and 17-61% of carcinoid tumors. TTF-1 is expressed in 100% of well differentiated and 90% poorly differentiated thyroid carcinomas. TTF-1 may also be expressed in some central nervous system neoplasms. TTF-1 is generally not expressed in squamous cell carcinoma, mesothelioma, and pancreatic carcinoma. There is a growing number of reports of aberrant TTF-1 staining detected in tumors previously considered TTF-1 negative, mainly due to use of highly sensitive clones. For example, positive TTF-1 staining has been reported in gastric carcinoma, breast carcinoma, colorectal adenocarcinoma, prostate adenocarcinoma, and some other neoplasms. TTF-1 is a useful aid for differentiating primary vs. metastatic tumors in lung and thyroid and for differentiating between primary lung adenocarcinoma and squamous cell carcinoma.

The CK5/6 and TTF-1 immunohistochemical antibody cocktail, which utilizes a brown DAB chromogen for cytoplasmic CK5/6 and the AP Red chromogen for nuclear TTF-1, is useful for differentiating lung squamous cell carcinoma (most often CK5/6 positive and TTF-1 negative) from lung adenocarcinoma (most often CK5/6 negative and TTF-1 positive) when used with a panel of other antibodies.

The Tissue-Tek Genie DUO anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail is a primary antibody cocktail containing mouse monoclonal anti-cytokeratin 5/6 antibody [D5/16B4] and rabbit monoclonal anti-TTF-1 [EP229] that recognize the human cytokeratin 5/6 and TTF-1 proteins respectively and is provided in buffered saline containing 1% bovine serum albumin and 0.09% sodium azide. FFPE specimen sections are placed on positively charged slides and the paraffin is removed using the Tissue-Tek Genie® Dewax Solution (REF 8865-G001), after which heat-induced epitope retrieval is performed using the Tissue-Tek Genie® High pH Antigen Retrieval Solution (REF 8744-G001).

IHC demonstration of CK5/6 and TTF-1 in FFPE specimen sections is achieved through use of the Tissue-Tek Genie DUO anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail and the Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit (8837). This procedure entails the sequential application of antibody and kit components as follows:

- Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit, Protein Block
- Tissue-Tek Genie® DUO anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail
- Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit, Link Mouse
- Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit, Link Rabbit
- Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit, Poly HRP + AP Conjugate
- Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit, DAB
- Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit, AP Red

Tissue-Tek Genie® Hematoxylin (REF 8830-M250) is then used to visualize the nuclei of cells. The IHC stained slide is cover-slipped and the FFPE specimen section reviewed using a light microscope.

## Expected results

Specificity and intended use of this antibody cocktail were validated by performing IHC staining on the Tissue-Tek Genie Advanced Staining System using FFPE normal and tumor specimen sections.

In normal tissues, CK5/6 brown cytoplasmic staining is observed in the basal cells of the respiratory epithelium, prostate glands, breast, skin, esophagus, and tonsil; CK5/6 brown cytoplasmic staining is also observed in various layers of squamous epithelium of tonsil and esophagus and in myoepithelial cells of breast and salivary gland acini. CK5/6 staining is not generally observed in the bile duct epithelial cells or hepatocytes. In neoplasms, CK5/6 brown cytoplasmic staining is observed in lung squamous carcinoma, other types of squamous carcinoma, and prostatic intraepithelial neoplasia (PIN). CK5/6 expression is generally not observed in adenocarcinoma of lung, breast, colon, stomach, thyroid, kidney, or germ cell tumors.

In normal tissues, TTF-1 red nuclear staining is observed in type II pneumocytes and other epithelial cells of lung and in follicular and parafollicular cells of thyroid gland. TTF-1 is not commonly observed in breast, colon, liver, tonsil, and other tissues. In neoplasms, TTF-1 is observed in lung adenocarcinoma, small cell carcinoma (including extrapulmonary types), and thyroid carcinoma. TTF-1 is generally not observed in lung squamous carcinoma. Positive TTF-1 staining is rarely observed in melanomas and gastrointestinal tumors.

Sensitivity and identification of CK5/6 and TTF-1 proteins by this antibody cocktail may be affected by improper specimen handling. This may alter antigenicity, weaken detection and may generate false negative results.

Cellular staining pattern: brown cytoplasmic staining for CK5/6 and red nuclear staining for TTF-1

Positive specimen control: lung, lung adeno and squamous carcinomas



## Cautions and warnings

For professional use only. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Wear protective gloves, clothing, and eye/face protection.

Capsules filled with ready-to-use, pre-diluted, antibody are for single use only. Do not attempt to refill or add additional reagent. Discard capsule after use.

Cartridges filled with ready-to-use, pre-diluted, antibody are intended for multiple uses. Do not attempt to refill or add additional reagent. Discard cartridge when empty.

It is recommended to include appropriate controls on each specimen slide to help in identifying any deviation that might occur during the staining process.

All disposal practices must be in compliance with all Federal, State/Provincial and local laws and regulations. Refer to the SDS for further information.

## Storage conditions

Store this product at 2-8°C.

## Instructions for use

Tissue-Tek Genie® DUO anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail, capsules (REF 8486-C010):

1. Place the Tissue-Tek Genie® Reagent Dispensing Area Tag (RDA-Tag) attached to the capsule into the RDA.
2. Push the capsule into the RDA with foil side down and click the attached RDA-Tag down into place on the RDA.
3. Place the RDA on the desired station of the Tissue-Tek Genie Advanced Staining System.
4. Place the slide with the specimen section on the same station, specimen section side down.
5. Assign protocol 8486 to the same station.
6. Initiate execution of protocol 8486.
7. The RDA-Tag 8486 will be scanned and registered automatically when the staining process is initiated.

8. During the primary antibody application step, the antibody will be released from the capsule into the RDA and onto the specimen section on the slide.

9. The staining protocol continues to the end.

Tissue-Tek Genie® DUO anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail, cartridge (REF 8486-M100):

1. Prior to placing the cartridge on the carousel of the Tissue-Tek Genie Advanced Staining System, prime the cartridge by facing the nozzle downwards and gently pinching the nozzle tubing until the tubing is filled with the reagent.
2. Place the cartridge on the carousel.
3. Click the RDA-Tag 8486 into place on the RDA.
4. Place the RDA on the desired station of the Tissue-Tek Genie Advanced Staining System.
5. Place the slide with the specimen section on the same station, specimen section side down.
6. Assign protocol 8486 to the same station.
7. Initiate execution of protocol 8486.
8. The RDA-Tag 8486 and the cartridge will be scanned and registered automatically when the staining process is initiated.
9. During the primary antibody application step, the antibody will be dispensed from the cartridge into the RDA and onto the specimen section on the slide.
10. The staining protocol continues to the end.

### Material required but not supplied

The following reagents may be required for staining but are not provided:

- Tissue-Tek Genie® Dewax Solution (REF 8865-G001)
- Tissue-Tek Genie® Wash Solution (REF 8874-G004)
- Tissue-Tek Genie® High pH Antigen Retrieval Solution (REF 8744-G001)
- Tissue-Tek Genie® DUO Non-immune Mouse and Rabbit Ig Antibody Cocktail, Negative Control (REF 8482-C010, 8482-M250)
- Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit (REF 8837-K250)

- Tissue-Tek Genie® Hematoxylin (REF 8830-M250)

Further information can be found on the Sakura Finetek USA website at [www.sakuraus.com/Genie](http://www.sakuraus.com/Genie)

## Order information

### Product code, product name and quantity

REF 8486-C010 Tissue-Tek Genie® DUO anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail, Ready-To-Use, 10 capsules; 1 pack.

REF 8486-M100 Tissue-Tek Genie® DUO anti-Cytokeratin 5/6 [D5/16B4] / TTF-1 [EP229] Antibody Cocktail, Ready-To-Use, 100 tests, 1 cartridge; 1 unit.

**NOTE:** The Safety Data Sheet (SDS) is available online on the Sakura Finetek USA website at [www.sakuraus.com/SDS.html](http://www.sakuraus.com/SDS.html)

## References

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## Contact

If located within the United States, contact Sakura Finetek USA, Inc. by calling toll free **1-800-725-8723** or contact your Sakura Finetek representative or authorized distributor.

In countries, other than the United States, contact the nearest authorized Sakura Finetek instrument distributor or representative. Contact details may be found at [www.sakura.com](http://www.sakura.com)



## Symbols

	Catalog number
	Batch code
	<i>in vitro</i> diagnostic medical device
	Temperature limitation
	Use by
	Manufacturer
	Consult instructions for use
	European Conformity
	Authorized representative in the European Community

Storage: 2°C  8°C







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