Tissue-Tek Genie® DUO

anti-p40 [BC28] / Napsin A [EP205] Antibody Cocktail

Instructions for use

Intended use

For *in vitro* diagnostic use.

Tissue-Tek Genie® DUO anti-p40 [BC28] / Napsin A [EP205] Antibody Cocktail is an antibody cocktail designed to qualitatively detect p40 and Napsin A 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

The ΔNp63 isoform, also known as p40, is the predominant isoform of p63 that is truncated, or lacking the N-terminal domain. p40 is a nuclear protein and a transcription factor. It is confined to basal cells of squamous epithelia and urothelium, as well as basal cells/myoepithelial cells in breast, sweat gland, salivary gland, and prostate. Recent studies have shown that p40 is highly specific for squamous and basal cells and is superior to p63 for diagnosing lung squamous cell carcinoma.

Napsin A is a protease that is predominantly expressed in the lung and kidney. It is expressed in alveolar type II cells. Napsin A is detected in 60-90% of non-mucinous lung adenocarcinoma and less frequently in mucinous lung adenocarcinoma and large cell carcinoma (20-30%). In most studies, Napsin A is not detected, or only focally staining, in lung squamous cell carcinoma. Studies have shown that Napsin A has approximately the same sensitivity as TTF1, but specificity is higher.

The Tissue-Tek Genie® DUO anti-p40 [BC28] / Napsin A [EP205] Antibody Cocktail utilizes the brown DAB chromogen for nuclear p40 and the AP Red chromogen for cytoplasmic Napsin A. The antibody cocktail is a useful aid in differentiating lung squamous cell carcinoma (most often p40 positive and Napsin A negative) from lung adenocarcinoma (most often p40 negative and Napsin A positive) when used with a panel of other antibodies.

The Tissue-Tek Genie® DUO anti-p40 [BC28] / Napsin A [EP205] Antibody Cocktail contains a mouse monoclonal anti-p40 antibody [BC28] and a rabbit monoclonal anti-Napsin A [EP205] antibody in a primary antibody cocktail that recognize the human p40 and Napsin A 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 p40 and Napsin A in FFPE specimen sections is achieved through use of the Tissue-Tek Genie® DUO anti-p40 [BC28] / Napsin A [EP205] Antibody Cocktail and the Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit (REF 8837-K250). 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-p40 [BC28] / Napsin A [EP205] 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 were validated by performing IHC staining on the Tissue-Tek Genie Advanced Staining System using FFPE normal and tumor specimen sections.

Normal tissue: p40 brown nuclear staining is observed in keratinocytes of the stratified squamous epithelium of skin, cervix, esophagus, and tonsil. p40 brown nuclear staining is observed in basal cells of respiratory epithelium of the bronchus and larynx, and in basal/myoepithelial cells of breast, sweat glands, salivary glands, and prostate. p40 brown nuclear staining is also seen in urothelial cells. In placenta, p40 brown nuclear staining is observed in dispersed cytotrophoblast cells. p40 staining is generally not observed in other cells.

Napsin A red granular cytoplasmic staining is observed in type II pneumocytes and alveolar macrophages of lung. Napsin A red granular cytoplasmic staining is observed in epithelial cells of proximal tubules of kidney. Napsin A staining is not observed in normal columnar epithelial cells and macrophages of the lamina propria of the colon.

Tumor tissue: p40 brown nuclear staining is observed in the neoplastic cells of lung squamous cell carcinoma. No p40 staining is observed in the neoplastic cells of lung adenocarcinoma. p40 brown nuclear staining is also observed in urothelial carcinomas and in squamous cell carcinomas of skin, esophagus, and cervix.

Napsin A red granular cytoplasmic staining is observed in the neoplastic cells of lung adenocarcinoma and renal cell carcinoma.

Sensitivity and identification of p40 and Napsin A 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 nuclear staining for p40 and red granular cytoplasmic staining for Napsin A

Positive specimen control: lung, lung adeno-carcinomas 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-p40 [BC28] / Napsin A [EP205] Antibody Cocktail, capsules (REF 8487-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 8487 to the same station.
6. Initiate execution of protocol 8487.
7. The RDA-Tag 8487 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-p40 [BC28] / Napsin A [EP205] Antibody Cocktail, cartridge (REF 8487-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 8487 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 8487 to the same station.
7. Initiate execution of protocol 8487.
8. The RDA-Tag 8487 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**

Order information

Product code, product name and quantity

REF 8487-C010 Tissue-Tek Genie® DUO anti-p40 [BC28] / Napsin A [EP205] Antibody Cocktail, Ready-To-Use, 10 capsules; 1 pack.

REF 8487-M100 Tissue-Tek Genie® DUO anti-p40 [BC28] / Napsin A [EP205] 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**

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**

Symbols

REF	Catalog number
LOT	Batch code
IVD	*in vitro* diagnostic medical device
	Temperature limitation
	Use by
	Manufacturer
	Consult instructions for use
CE	European Conformity
EC REP	Authorized representative in the European Community

Storage: 2°C 8°C

IVD

CE

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