

Tissue-Tek Genie® DUO

anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN
Antibody Cocktail

Instructions for use

Intended use

For *in vitro* diagnostic use.

Tissue-Tek Genie® anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN Antibody Cocktail is an antibody cocktail designed to qualitatively detect AMACR, p63, and cytokeratin 5/6 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 cocktail on the Tissue-Tek Genie Advanced Staining System, using Tissue-Tek Genie® reagents, specifically the Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit, and FFPE specimen sections. Staining quality may diminish when used with other systems and/or reagents.

Summary and principle

AMACR (Alpha Methylacyl Coenzyme A Racemase), also known as P504S, is a mitochondrial enzyme expressed in many normal epithelia such as hepatocytes, tubular epithelia of kidney, epithelia of gall bladder, bronchial epithelium of lung, and colonic

surface epithelium. AMACR is often expressed at high levels in the cytoplasm of the cells comprising high-grade prostatic intraepithelial neoplasia (HG-PIN) and prostate adenocarcinoma. AMACR expression is commonly low or undetectable in normal and hyperplastic prostate glands.

P63 is a transcription factor and plays a critical role in growth and development of many epithelial tissues. P63 is expressed in basal cells of squamous epithelia including epidermis and hair follicles, basal cells of urothelium and prostate, and myoepithelial cells of breast, sweat glands, and salivary glands.

Cytokeratin 5 and 6, are high molecular weight cytokeratin with cytokeratin 5 (CK5) expressed in various epithelial basal cells and mesothelial cells, and cytokeratin 6 (CK6) expressed in proliferating squamous epithelium.

P63 is expressed in the nucleus and CK5/6 in the cytoplasm of basal cells of prostate glands. Prostatic intraepithelial neoplasia (PIN) is a condition defined by neoplastic growth of epithelial cells within preexisting benign prostatic acini or ducts. PIN is a non-invasive precursor to prostate adenocarcinoma and demonstrates preservation of basal cells. In contrast, prostate adenocarcinoma is characterized by a complete lack of basal cells. The immunohistochemical combination of nuclear p63 expression and cytoplasmic CK5/6 expression aids in detection of peripheral basal cells while cytoplasmic expression of AMACR in glandular cells aid in the detection of

potentially neoplastic proliferation. This antibody cocktail, which utilizes a brown DAB chromogen for p63 and CK5/6 and the AP Red chromogen for AMACR, is useful for distinguishing benign and malignant proliferations in the prostate, when used in a panel with other antibodies.

The Tissue-Tek Genie anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN Antibody Cocktail is a primary antibody cocktail against the human AMACR, p63, and cytokeratin 5/6 proteins 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 AMACR, p63, and cytokeratin 5/6 in FFPE specimen sections is achieved through use of the Tissue-Tek Genie anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN 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 one antibody cocktail and kit components as follows:

- Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit, Protein Block
- Tissue-Tek Genie® anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN 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.

AMACR red granular cytoplasmic staining is observed in the epithelial cells of the renal tubules and colon. AMACR red staining is not observed or is weak and focal in the epithelial cells of benign and hyperplastic prostate glands. AMACR red staining is not observed in tonsil. AMACR red granular cytoplasmic staining is observed in the epithelial cells of prostate intraepithelial neoplasia (PIN) and of prostate adenocarcinomas.

P63 brown nuclear staining is observed in basal cells of normal prostate gland and of PIN. P63 brown nuclear staining is also observed in the dispersed cytotrophoblast cells of placenta, benign and malignant squamous epithelial cells including squamous cell carcinomas, and scattered lymphocytes of tonsil.

CK5/6 brown cytoplasmic staining is observed in the basal cells of normal prostate glands and of PIN. CK5/6 brown cytoplasmic staining is also seen in layers of squamous epithelial cells of tonsil and esophagus as well as squamous cell carcinomas. CK5/6 brown cytoplasmic staining is not observed in the bile duct epithelial cells and is not observed in the neoplastic cells of breast ductal carcinomas.

Sensitivity and identification of AMACR, p63, and cytokeratin 5/6 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: Cytoplasmic for AMACR, nuclear for p63, and cytoplasmic for CK5/6

Positive specimen control: Normal (benign) prostate and prostate adenocarcinoma

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® anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN Antibody Cocktail, capsules (REF 8485-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 8485 to the same station.
6. Initiate execution of protocol 8485.
7. The RDA-Tag 8485 will be scanned and registered automatically when the staining process is initiated.
8. During the primary antibody application step, the antibody cocktail 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® anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN Antibody Cocktail, cartridge (REF 8485-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 8485 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 8485 to the same station.
7. Initiate execution of protocol 8485.
8. The RDA-Tag 8485 and the cartridge will be scanned and registered automatically when the staining process is initiated.
9. During the primary antibody application step, the antibody cocktail 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 8485-C010 Tissue-Tek Genie® anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN Antibody Cocktail, Ready-To-Use, 10 capsules; 1 pack.

REF 8485-M100 Tissue-Tek Genie® anti-AMACR [13H4] / p63 [MX013] / CK5/6 [D5/16B4] PIN 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

	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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