Tissue-Tek Genie® DUO

Mouse-DAB/Rabbit-AP Red Dual Detection Kit

REF 8837-K250

Instructions for use

For *in vitro* diagnostic use.

Intended purpose

Intended use: The Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit is designed to detect mouse and rabbit primary antibody cocktails in formalin-fixed, paraffin embedded (FFPE) human tissue specimen sections by immunohistochemistry (IHC) staining using the automated Tissue-Tek Genie® Advanced Staining System.

This device is an accessory to an *in vitro* medical device which must be used by a qualified pathologist as an aid to diagnosis to determine the pathological state of a patient.

The clinical interpretation must be made by a qualified pathologist, in conjunction with histological examination, relevant clinical information, other diagnostic tests, and proper controls.

Limitations

This product has been designed to optimize staining quality of IHC assays using Tissue-Tek antibodies and reagents in FFPE human specimen sections on the Tissue-Tek Genie Advanced Staining System. Staining quality may diminish when used with other systems and/or reagents.

When using frozen sections or cell smears, the optimal procedure must be determined and verified by the user.

The recommended protocol is determined by the specific Tissue-Tek Genie antibody.

Summary and principle

Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit is a visualization system for IHC applications.

IHC staining is an established in vitro diagnostic method to visualize the presence of specific proteins expressed by the tissue specimen within a tissue section to study the microscopic features.

IHC staining is accomplished in two steps:

1) a primary antibody recognizes a particular target protein expressed on a specific cell compartment of a specific cell type on various tissues, and

2) a secondary and tertiary antibody conjugated to a chromogenic or fluorescent enzyme binds with the primary antibody for the detection of the antibody-antigen interaction. In chromogenic detection under a light microscope, an enzyme conjugated to the antibody cleaves a substrate to produce a colored precipitate at the location of the protein. In fluorescent detection, a fluorophore conjugated to the antibody is visualized using fluorescence microscopy.

To prepare for IHC staining, FFPE specimen sections are placed on positively charged slides. 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) or the Tissue-Tek Genie® Citrate pH 6 Antigen Retrieval Solution (REF 8742-G001). IHC demonstration is achieved through the use of specific primary antibodies and the Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit

The Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit uses a non-biotin-based system to detect and to visualize mouse and rabbit primary antibodies bound to antigens in FFPE specimen sections. The detection system uses a protein block to prevent nonspecific binding of antibodies to antigens with downstream components of the detection system. After blocking, Tissue-Tek Genie® DUO antibody cocktail is applied on the tissue sections simultaneously using prefilled capsules or cartridges. Subsequently, the detection system sequentially applies the Link Mouse solution that binds to mouse primary antibodies and then the Link Rabbit solution that binds to rabbit primary antibodies. This is followed by an application of a cocktail containing a horseradish peroxidase (HRP) conjugated tertiary antibody for mouse and an alkaline phosphatase (AP) conjugated tertiary antibody for rabbit. The resulting complex is visualized by sequentially applying the diamino-benzidine (DAB) substrate-chromogen solution, and then by applying the AP Red substrate-chromogen solution, which create colored precipitates at the location of the antigens. These precipitations can be seen through a light microscope.

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

Expected results

Analytical sensitivity/specificity: Positive staining of antigens detected by mouse primary antibodies is indicated by a brown to dark brown color. Positive staining of antigens detected by rabbit primary antibodies is indicated by a red color. The staining profile is determined by the primary antibody.

Positive and negative control specimens should be run simultaneously with unknown specimens. Brown and red staining should be present on the positive control specimen at the expected localization of the target antigen. If non-specific staining is present, this will be recognized as a diffuse, pink/red and/or brown staining on the slides treated with the negative control reagent.

Repeatability and reproducibility are demonstrated for each manufacturing lot by functional testing.

As a standalone reagent, this product cannot be tested for diagnostic sensitivity and specificity. This reagent must be used in conjunction with a Tissue-Tek Genie IHC antibody (REF 8XXX) as well as the other reagents associated with the Tissue-Tek Genie® Advanced Staining System. Refer to the instructions for use (IFU) of the Tissue-Tek Genie IHC antibody used with this reagent for the expected patient sample results.

If any unexpected staining cannot be attributed to variations in laboratory procedures and a problem with the detection reagents is suspected, contact your local distributor or the regional office of Sakura Finetek.

Cautions and warnings

For professional use, only.

The DAB reagent, AP Red reagent, and intensifier are harmful if swallowed or in contact with skin. The DAB reagent is suspected of causing genetic defects and may cause cancer. Do not handle until all safety precautions have been read, understood and implemented. Do not eat, drink or smoke when using this product. Wear protective gloves, clothing, and eye/face protection. IF SWALLOWED or ON SKIN: call a POISON CENTER. If exposed or concerned, get medical advice.

Reagents contain material of animal origin. As with any product derived from biological sources, take reasonable precautions when handling. Avoid contact with eyes, skin, and mucous membranes. Wear protective gloves, clothing, and eye/face protection.

Avoid release to the environment. The DAB intensifier is toxic to aquatic life. All disposal practices must be in

compliance with all Federal, State/Provincial and local laws and regulations. Refer to the SDS for further information.

The AP red chromogen reagent is soluble in alcohol. DO NOT use extended alcohol baths or extended use of xylene to dehydrate and clear slides. Refer to the instructions for automated coverslipping section.

Slides stained with AP red chromogen should be stored away from direct light.

Cartridges filled with ready-to-use reagents are for multiple use, up to the number of tests specified. Do not attempt to re-fill or add additional reagent. Discard cartridge when empty.

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

Specimen collection and preparation for analysis

Routinely processed, formalin-fixed, paraffin embedded tissue specimens are suitable for use with Tissue-Tek Genie reagents and a Tissue-Tek Genie Advanced Staining System (see section "Material required but not supplied"). The recommended tissue fixation is performed using 10% (v/v) neutral buffered formalin for 24-72 hours. Variable results may occur because of prolonged fixation or special processes such as decalcification of bone marrow preparations. Each cut section should be 3-5 µm in thickness and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 30 minutes to overnight in a 60°C ± 2°C oven.

Storage conditions

Store this product at 2-8°C. Do not freeze. Return to 2-8°C after use.

For the date of expiration, refer to the label on the product.

The reagent will be stable until its expiration date when stored and handled properly. Do not use the reagent beyond its assigned expiration date. Storage conditions other than those specified above must be verified by the user.

Do not use when precipitate is present and visible in the reagent.

Instructions for use

1. Prior to placing cartridges of this kit on the carousel of the Tissue-Tek Genie Advanced Staining System, prime each cartridge by facing the nozzle downwards and gently pinching the nozzle tubing until the tubing is filled with the reagent.
2. Place each cartridge on the carousel. In clear view of the cartridge holders in the carousel, seat each cartridge by aligning the back of it with the back guide of the cartridge holder and fully inserting the silicone tubes and nozzles into the cartridge insertion area.
3. The cartridges will be scanned and registered automatically when a staining run is initiated or the "SCAN REAGENTS" process is started.
4. The 6 cartridges of this kit are registered with the software as one kit. Do not replace any reagent of a kit with the corresponding reagent from another kit.

Refer to the Tissue-Tek Genie Advanced Staining System operating manual for more details. Refer to the Instruction for Use of each specific Tissue-Tek Genie primary antibody and all other Tissue-Tek Genie products.

Material required but not supplied

- Tissue-Tek SmartWrite® Frosted Slides-Charged (REF 9036, REF 9046, REF 9048, REF 9050, REF 9052, REF 9054)
- Drying oven capable of maintaining a temperature of 60°C ± 2°C
- Tissue-Tek Genie® Reagent Dispense Area [RDA] (REF 8616-G090)
- Tissue-Tek Genie® Dewax Solution (REF 8865-G001)
- Tissue-Tek Genie® Wash Buffer Solution (REF 8874-G004)
- Tissue-Tek Genie® High pH Antigen Retrieval Solution (REF 8744-G001)

- Tissue-Tek Genie® DUO antibody Cocktail (REF 8XXX-XXXX)
- Tissue-Tek Genie® DUO Non-Immune and Rabbit Ig Antibody Cocktail (REF 8482-C010 or REF 8482-M250)
- Tissue-Tek Genie® Hematoxylin (REF 8830-M250)

Instructions for automated coverslipping

1. Ensure the Tissue-Tek Prisma® / Tissue-Tek Prisma® Plus Automated Slide Stainer (REF 6130/6170) linked to a Tissue-Tek Film® Automated Coverslipper / Tissue-Tek Glas™ g2 Automated Glass Coverslipper (REF 4740/6500) are operational.
2. Transfer the slides from the Tissue-Tek Genie Advanced Staining System promptly into a Tissue-Tek® Slide Basket (REF 4768) and place the basket immediately into a start station on the Tissue-Tek Prisma® / Tissue-Tek Prisma® Plus Automated Slide Stainer.
3. Start the Sakura Finetek validated IHC dehydration program listed below. This program must be set to Priority (refer to the Tissue-Tek Prisma® / Tissue-Tek Prisma® Plus Automated Slide Stainer operating manual).

Step	Station	Solution	Time	Delay	Mix
1	S*	Start Station	--:--:--		
2	W*	Wash Station	00:01:00	==	EW
3	23	Alcohol 95%	00:00:10	==	ON
4	22	Alcohol 100%	00:00:10	==	ON
5	21	Alcohol 100%	00:00:10	==	ON
6	19	Xylene	00:00:10	==	ON
7	18	Xylene	00:00:10	==	ON
8	17	Xylene	00:00:10	==	ON
9	E1-*	End Station 1	--:--:--		

Further information can be found on the Sakura Finetek USA website at **www.sakuraus.com/Genie**

Troubleshooting

Testing runs should include proper reagent and tissue controls.

- If the positive control exhibits negative, weaker, or stronger staining, or more background staining than expected, other positive controls on the same instrument run should be examined to determine if this is due to the antibody, other reagents, software, instrumentation, or the processing and fixation of tissue specimen(s).
- If the paraffin has not been removed completely, the deparaffinization procedure should be verified.
- If tissue sections have washed off, slides should be examined to ensure that they are positively charged, and the specimen should be examined for possible inadequate processing or fixation.
- Refer to the Tissue-Tek Genie Advanced Staining System operating manual or contact your Sakura Finetek Technical support representative for information or assistance.

Order information / product provided

Product code, product name and quantity

REF 8837-K250 Tissue-Tek Genie® DUO Mouse-DAB/Rabbit-AP Red Dual Detection Kit; RTU, cartridge containing 250 tests, 1 each, Protein Block, Link 1, Link 2, Poly HRP + AP Conjugates, DAB, AP Red/kit.

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

References

1. Magaki, S., Hojat, S.A., Wei, B. et al. (2019). An Introduction to the Performance of Immunohistochemistry. Methods Mol Biol. 1897, pp 289-298.
2. Lin, F. and Chen, Z. (2014). Standardization of Diagnostic Immunohistochemistry: Literature Review and Geisinger Experience. Arch Pathol Lab Med. 138, pp 1564-1577.
3. Duraiyan, J., Govindarajan, R., Kaliyappan, K., et al., (2012). Applications of immunohistochemistry. J Pharm Bioallied Sci., 4, pp S307-S309.
4. Rudiger, T., Hofler H, Kreipe HH., et al. (2002). Quality assurance in immunohistochemistry: results of an interlaboratory trial involving 172 pathologists. Am J Surg Pathol. 26(7), pp 873-882.

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**

Any incident should be reported to the manufacturer. In the European Union, any serious incident can also be reported to a competent authority of the appropriate Member State.

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

LOT

Please see product label for lot information and expiration date, and the date of manufacture, if available

Storage: 2°C 8°C

IVD

CE

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	Made in U.S.A.

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