Tissue-Tek Genie®

Hematoxylin

REF 8830-M250

Instructions for use

For *in vitro* diagnostic use.

Intended purpose

Intended use: The Tissue-Tek Genie® Hematoxylin is designed to counterstain cellular nuclei in formalin-fixed, paraffin embedded (FFPE) human specimen sections after immunohistochemistry (IHC) staining of specific protein targets or chromogenic *in situ* hybridization (CISH) of specific DNA or RNA targets 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 the staining quality of IHC or CISH assays using Tissue-Tek Genie® antibodies, probes, and reagents in formalin-fixed, paraffin embedded (FFPE) human tissue specimen sections on the Tissue-Tek Genie Advanced Staining System. Staining quality may diminish when used with other systems and/or reagents.

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

Summary and principle

The Tissue-Tek Genie Hematoxylin is an ancillary reagent after IHC or CISH staining on the Tissue-Tek Genie Staining System. Tissue-Tek Genie Hematoxylin stains nuclei of cells in specimen sections blue in contrast to antigen staining.

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

IHC or CISH staining is accomplished in two steps:

1) a primary antibody or probe recognizes a particular target protein or nucleic acids 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 or probe for the detection of the antibody-antigen or probe-nucleic acid 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 or CISH 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 Tissue Tek Genie® Citrate pH 6 Antigen Retrieval Solution (REF 8742-G001). IHC demonstration is achieved through the use of a specific primary antibody and the Tissue-Tek Genie® Detection Kit, DAB (REF 8826-K250). CISH demonstration is achieved using a specific probe and the Tissue-Tek Genie® Amplifier (REF 9808-M100) or a user-defined CISH amplification linker antibody, prepared and filled by the user into the Tissue-Tek Genie® User-Fillable Cartridge (REF 9809-G006).

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

Nuclei of cells are blue.

Cellular staining pattern: N/A

Positive tissue control: N/A

As a standalone reagent, this product cannot be tested for sensitivity and specificity. This reagent must be used in conjunction with a Tissue-Tek Genie IHC antibody (REF 8XXX) or CISH probe (REF 9XXX) or a Tissue-Tek Genie® User-Fillable Capsule filled with primary antibody or CISH probe as well as the other reagents associated with Tissue-Tek Genie® Advanced Staining System. Refer to the instructions for use (IFU) of the Tissue-Tek Genie IHC antibody or CISH probe or package insert (PI) of the IHC antibody or CISH probe used with this reagent for the expected patient sample results.

It is recommended that the performance of the reagents and system are verified by staining of appropriate tissue controls.

The clinical interpretation of the stained tissue must be made by a qualified pathologist.

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.

Cartridges filled with ready-to-use reagents are intended for multiple uses. Do not attempt to re-fill or add additional reagent. Discard the cartridges when empty.

It is recommended to include appropriate controls, onto 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.

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 the product at room temperature 15-30°C.

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 the Tissue-Tek Genie Hematoxylin cartridge on the carousel of the Tissue-Tek Genie Advanced Staining System, prime this cartridge by facing the nozzle downwards and gently pinching the nozzle tube until the tube 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 cartridge will be scanned and registered automatically when a staining protocol is initiated.

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® Dewax Solution (REF 8865-G001)
- Tissue-Tek Genie® Wash Buffer Solution (REF 8874-G004)
- Tissue-Tek Genie® Citrate Antigen Retrieval Solution (REF 8742-G001)
- Tissue-Tek Genie® User-Fillable Capsule Sealing System (REF 8610-K050)
- Tissue-Tek Genie® Reagent Dispense Area [RDA] (REF 8616-G090)
- Tissue-Tek Genie® Pro Detection Kit, DAB (REF 8826-K250)

Required for IHC applications:

- Tissue-Tek Genie® Primary Antibodies (REF 8XXX-XXXX) or primary antibody, ready-to-use
- Tissue-Tek Genie® Non-Immune Ig Negative Control (mouse antibody REF 8604-C010 or REF 8604-M250) (rabbit antibody REF 8605-C010 or REF 8605-M250) (DUO mouse and rabbit antibody cocktail REF 8482-C010 or REF 8482-M250)
- Tissue-Tek Genie® High pH Antigen Retrieval Solution (REF 8744-G001)
- Tissue-Tek Genie® Pro Antibody Diluent (REF 8866-G004)
- Tissue-Tek Genie® Detection Kit (AP, REF 8836-K250 or DUO, REF 8837-K250)
- Tissue-Tek Genie® User-Fillable Cartridge for Biomarker-Binding Reagent (REF 8622-G006)

Required for CISH applications:

- Tissue-Tek Genie® CISH probe (REF 9XXX-XXXX) or CISH probe, ready-to-use
- Tissue-Tek Genie® CISH mRNA Negative Control Probe (REF 9860-C010)
- Tissue-Tek Genie® CISH mRNA Positive Control Probe (REF 9861-C010)
- Tissue-Tek Genie® Proteinase K (REF 9811-M100)
- Tissue-Tek Genie® SSC Stringent Wash Buffer (REF 9810-G001), Genemed® SSC Stringent Wash Buffer (REF 10-0176), or SSC stringent wash buffer, ready-to-use
- Tissue-Tek Genie® CISH Amplifier (REF 9808-M100)
- Tissue-Tek Genie® User-Fillable Cartridge for CISH Amplification Linker (REF 9809-G006) to be filled with CISH amplification linker antibody

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

Troubleshooting

Testing run 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 primary antibody, probe, capsule, 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 confirm 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 8830-M250 Tissue-Tek Genie® Hematoxylin; RTU, cartridge containing 250 tests.

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

References

1. Duraiyan, J., Govindarajan, R., Kaliyappan, K., et al., (2012). Applications of immunohistochemistry. J Pharm Bioallied Sci., 4, pp S307-S309.
2. 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.
3. Magaki, S., Hojat, S.A., Wei, B. et al. (2019). An Introduction to the Performance of Immunohistochemistry. Methods Mol Biol. 1897, pp 289-298.
4. Rimsza, LM., Day, WA., McGinn, SM., et al. (2014). Kappa and Lambda light chain mRNA in situ hybridization compared to flow cytometry and immunohistochemistry in B cell lymphomas. Diagnostic pathology. 9, pp144.

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: 15°C 30°C

IVD

CE

	Sakura Finetek USA, Inc. 1750 W 214th Street Torrance, CA 90501 U.S.A.
EC REP	Sakura Finetek Europe B.V. Flemingweg 10a 2408 AV Alphen aan den Rijn The Netherlands
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