Tissue-Tek Genie® CISH

EBER Probe

REF 9852-C010

Instructions for use

For *in vitro* diagnostic use.

Intended purpose

Intended use: The Tissue-Tek Genie® CISH EBER Probe is intended for chromogenic *in situ* hybridization (CISH) using the automated Tissue-Tek Genie® Advanced Staining System in conjunction with Tissue-Tek Genie® Pro Detection Kit, DAB, and other Tissue-Tek Genie® bulk and ancillary reagents to qualitatively detect cells expressing Epstein-Barr virus (EBV)-encoded RNA (EBER) in routine formalin-fixed, paraffin embedded (FFPE) human tissue sections by light microscopy.

The Tissue-Tek Genie CISH EBER Probe functions as an aid to diagnose and is useful for the identification of cells infected with Epstein-Barr virus (EBV), including those found in certain types of EBV-related lymphoproliferative conditions, when used with a panel of other antibodies and/or CISH probes.

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

The Tissue-Tek Genie® CISH EBER Probe has been optimized for use with the Tissue-Tek Genie® Advanced Staining System, Tissue-Tek Genie® Citrate Antigen Retrieval Solution (REF 8742-G001), Tissue-Tek Genie® Proteinase K (REF 9811-M100), Tissue-Tek Genie® Pro Detection Kit, DAB (REF 8826-K250), Tissue-Tek Genie® CISH Amplifier (REF 9808-M100), other Tissue-Tek Genie® reagents, and FFPE specimen sections. Staining quality may be diminished when used with other systems and/or reagents.

Protocol time may vary due to variation of tissue type, fixation, and processing. The incubation time of the Tissue-Tek Genie® Proteinase K may differ depending on tissue processing and fixation condition.

Staining quality may be diminished by improper or incomplete removal of the paraffin.

Special processing of tissues such as decalcification of bone marrow tissues may lead to inconsistent staining.

Positively charged slides are recommended to obtain optimal staining with the Tissue-Tek Genie Advanced Staining System.

In rare cases, light membranous and cytoplasmic staining can be observed in scattered cells in tonsils, which should not be considered as positive staining.

Summary and principle

In situ hybridization (ISH) staining is an established in vitro diagnostic method to visualize the presence or absence of particular nucleic acid sequences (i.e., RNA or DNA) within a tissue section. Specifically,

Chromogenic ISH (CISH) uses labels which are detectable by bright-field microscopy.

Epstein-Barr virus (EBV), a double-stranded DNA virus, is a member of the Herpes virus family and causes both lytic and latent infections. Latent EBV infection is associated with several conditions including Hodgkin lymphoma (30-50%), Burkitt lymphoma, nasopharyngeal carcinoma, lymphoproliferative disorders, and lymphoma associated with immunosuppression including transplant and AIDS. EBV is also detected in some gastric cancers and some T-cell lymphomas. Latency is established in resting memory B-lymphocytes where only a small portion of the viral genome is transcribed. EBER1 and EBER2 are small non-coding RNAs localized in the nucleus of human cells infected with EBV and are transcribed by host RNA polymerase III during latent infection. EBER1 and EBER2 are reliably expressed at high levels, around one million copies per cell, in virtually all latently infected cells in benign and malignant lesions. EBER detection by CISH is a sensitive method for the detection of latent EBV infection.

The Tissue-Tek Genie CISH EBER Probe contains a cocktail of FITC-labelled single-stranded DNA oligonucleotide and is used for the detection of EBER transcripts in formalin-fixed, paraffin-embedded (FFPE) tissue sections by CISH. This EBER probe binds specifically to EBER1 and EBER2. The probe does not detect other DNA or RNA sequences.

The Tissue-Tek Genie CISH EBER Probe (REF 9852-C010) is provided as a Ready-to-Use (RTU) hybridization solution in a citrate-based SSC buffer containing formamide and dextran sulfate. 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 the tissue section is pretreated using the Tissue-Tek Genie® Citrate Antigen Retrieval Solution (REF 8742-G001) and Tissue-Tek Genie® Proteinase K (REF 9811-M100). The hybridization of the Tissue-Tek Genie® CISH EBER Probe at 37°C for 45 minutes is followed by a stringent wash with Tissue-Tek Genie® CISH SSC Stringent Wash Buffer (REF 9810-G001) and Tissue-Tek Genie® Wash Buffer Solution (REF 8874-G004). The Tissue-Tek Genie® CISH Amplifier (REF 9808-M100), a rabbit anti-FITC antibody, is added as a primary antibody, allowing chromogenic detection and visualization of specific nucleic acids in FFPE tissues sections. Tissue-Tek Genie® Pro Detection Kit, DAB (REF 8826-K250) is then used to detect and visualize the rabbit anti-FITC antibody with a non-biotin-based system. The Tissue-Tek Genie® Hematoxylin (REF 8830-M250) counterstain is then used to visualize the nuclei of cells. The CISH stained slide is coverslipped and the FFPE specimen section is reviewed using a light microscope.

The total turnaround time of the assay is 2 hours 33 minutes.

Expected results

Cellular staining pattern: nuclear

Positive tissue control: EBV positive lymphoma, mononucleosis

Analytical sensitivity/specificity: Analytical specificity and sensitivity were demonstrated by performing CISH staining on the Tissue-Tek Advanced Staining System using normal and neoplastic FFPE tissue sections as follows:

In normal tissues without EBV infection, no staining was observed. In neoplastic tissues, positive nuclear staining was observed in Reed-Sternberg cells of Hodgkin lymphomas, diffused large B cell lymphomas, neoplastic cells of Burkitt lymphoma, nasopharyngeal carcinoma, and extranodal NK/T lymphoma.

The repeatability and reproducibility of this probe were demonstrated by performance tests using various lots, instruments, staining stations, operators, and specimens.

Diagnostic sensitivity/specificity: Diagnostic sensitivity was demonstrated by positive nuclear staining in 3 of 3 EBV infected mononucleosis, 2 of 3 hyperplasia, and 6 of 14 Hodgkin lymphomas.

Diagnostic specificity was demonstrated by the lack of positive nuclear staining in other types of neoplastic cells including those of breast, lung, intestine, prostate, brain, and skin origin.

Cautions and warnings

For professional use only.

The probe contains **formamide** and is labelled **Danger**.

The probe may cause cancer, damage fertility or the unborn child, and damage organs through prolonged or repeated exposure.

Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking.

The material and/or its container must be disposed of as hazardous waste. Avoid release into the environment.

Use personal protective equipment as required. Obtain special instruction before use. Do not handle until all safety precautions have been read and understood. Wear protective gloves, protective clothing, and eye protection.

Wash hands and surfaces thoroughly after handling this product. Do not eat, drink, or smoke when using this product. Do not breathe mist/vapors/spray.

Remove contaminated clothing. Wash contaminated clothing before reuse. If on skin: Wash off with soap and plenty of water. If ingested: call a poison center and consult a physician. Do NOT induce vomiting. If the product enters the eye: flush eyes with plenty of water and consult a physician. In case of fire: Use alcohol-resistant foam, dry chemical or carbon dioxide fire extinguishers.

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

Capsules filled with reagent are for single use.

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

As the probe is designed to detect mRNA, it is important to avoid contamination of tissue specimen with RNase prior to the probe hybridization.

Specimen collection and preparation for analysis

Routinely processed, formalin-fixed, paraffin embedded tissues are suitable for use with this reagent when used 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 due to 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. Slides should be stained immediately, as the quality of nucleic acid targets in tissue sections may diminish over time.

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

Tissue-Tek Genie® CISH EBER Probe, RTU, 10 capsules/pack (REF 9852-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 9852 to the same station.
6. Initiate execution of protocol 9852.

7. The RDA-Tag 9852 will be scanned and registered automatically when the staining process is initiated.
8. During the CISH EBER Probe application step, the probe 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.

Material required but not supplied

- Positive and negative tissue controls
- Tissue-Tek Genie® CISH mRNA Positive Control Probe (REF 9861-C010)
- Tissue-Tek Genie® CISH mRNA Negative Control Probe (REF 9860-C010)
- 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® Proteinase K (REF 9811-M100)
- Tissue-Tek Genie® CISH SSC Stringent Wash Buffer (REF 9810-G001)
- Tissue-Tek Genie® CISH Amplifier (REF 9808-M100)
- Tissue-Tek Genie® Pro Detection Kit, DAB (REF 8826-K250)
- Tissue-Tek Genie® Hematoxylin (REF 8830-K250)
- Tissue-Tek Genie® Reagent Dispense Area [RDA] (REF 8616-G090)

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, or 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 probe, 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 9852-C010 Tissue-Tek Genie® CISH EBER Probe; RTU, 10 capsules/pack

This product is not available in the U.S.A.

NOTE: The Safety Data Sheet (SDS) is available online on the Sakura 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**

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 and expiration date information and if available the date of manufacture

Storage: 2°C 8°C

IVD

CE

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GS-33498 Rev. B, 2022-03
TE-13-031-01 v1.0