RISH™ Epstein-Barr Encoded RNA (EBER) Probe

Hybridization Probe
902-RI0001-110322

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Predilute	RI 0001 T	20 tests	Ready-to-use	N/A
ONCORE Pro	OPPR 0001 T60	60 tests	Ready-to-use	N/A

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

Summary & Explanation:
The Epstein – Barr virus (EBV) is a member of the gamma-herpes viruses (HHV-4). It is a linear 184,000 base pair double stranded DNA virus. It was the first oncogenic virus to be discovered.[1] Infection by this virus can show signs of a slight viral infection or it can be present as Infectious Mononucleosis. The most common target cells for the Epstein-Barr virus are the B lymphocytes and the nasopharyngeal epithelial cells. The Epstein-Barr virus massively infects the human population and sero-epidemiological studies show that 90% of adults have been infected by this virus.[2] Latently infected B lymphocytes express abundantly (104-105 copies), among other genes, a short nonpolyadenylated chain of RNA that does not transduce to a protein, consisting of two fragments known as EBER 1 and EBER 2. The expression of EBER (Epstein-Barr virus encoded RNAs) is nuclear. Although the function of EBER is unknown, it is believed that it may play a role in virus-produced oncogenesis.[3] There are numerous human tumors associated with EBV. These range from non-differentiated nasopharyngeal carcinoma to African Burkitt's lymphoma, Hodgkin's disease mixed cellularity, some B, T and NK lymphomas, as well as in lymphoproliferative processes associated with immunodeficiency.[4]

Species Reactivity:
EBER RNA in reactive and tumoral cells.

Cellular Localization: Nuclear

Known Applications:
in situ hybridization (formalin-fixed paraffin-embedded tissues)

Supplied As:
RI0001
(20 tests at approximately 20 µL per test) is provided in hybridization buffer containing dextran sulfate and nucleic acid carriers.

OPPR0001
(60 tests at approximately 130 µL per test) is provided in hybridization buffer containing dextran sulfate and nucleic acid carriers.

Reconstitution, Dilution and Mixing:
Products are supplied as ready-to-use DNA probe in hybridization Buffer. No reconstitution, dilution or mixing required.

Storage and Stability:
Store probe at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Materials and Reagents Required but Not Provided:
Microscope slides, positively charged
Desert Chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
ISH Dewax Kit (OPRI6020K)*
Decloaking Chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*(TBS)

Materials and Reagents Required but Not Provided Cont'd:
RISH™ Retrieval Solution (RI0209M) *
ISH Retrieval (AR3) (OPRI6021) *
ISHzyme Kit (OPRI6039K)*
Peroxidase block*
Probe Enhancer (RNA) (OPRI6024) *
Negative control reagents*
ISH Detection Reagents*
Chromogens*
Hematoxylin*
Bluing reagent*
Mounting medium*
IQ Aqua Sponge*
HybriSlip™ (or equivalent) *
IQ Kinetic Slide Stainer* or other hybridization oven
ONCORE Pro Automated Slide Stainer*

* Refer to the Biocare Medical website located at http://biocare.net for information regarding catalog numbers and ordering. Certain reagents listed above are based on specific application and detection system used.

Staining Protocol Recommendations (Manual Use):
Refer to RISH™ Detection Kit (RI0207KG or RI0213KG) datasheet for specific protocol recommendations.

Technical Notes:
1. This test should be performed on tissue sections where the presence of Epstein-Barr Virus is anticipated. 4-5 micrometer (µm) sections are sufficient to conduct this study. Preferably, the sections should be fresh and no more than 30 days old.
2. This DNA probe has been standardized using Biocare's IQ Kinetic Slide Stainer for hybridization and post-hybridization detection steps. Detection steps can also be programmed on an automated staining system.
3. If using commercially available humidity chambers, hybridize probe for 30-60 minutes. Both incubator and humidity chamber must be at 55ºC when hybridizing probe. Other hybridization chambers can be used, but measures should be taken to ensure that chamber is hermetically sealed during hybridization.
4. If a Decloaking Chamber™ or pressure cooker is not available, consider using a water bath or hot plate for retrieval. Place RISH™ Retrieval (1X) in glass (Pyrex) container and heat solution until the appropriate temperature is achieved (90°C). Heat slides in this solution for 15 minutes. Remove slides after incubation and immediately wash in distilled water. Proceed with probe hybridization.
5. The IQ Stainer can be used as an incubation and humidity chamber by using the IQ Aqua Sponge. Saturate IQ Aqua Sponge with distilled water, and place on hot bar set to 55°C for hybridization. Use the clear plastic hood to contain heat and moisture.
6. If probe appears cloudy, briefly vortex and heat to hybridization temperature (55°C) before application.
Note: The use of probe in amounts less than recommended may lead to inconsistent results.

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Staining Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

The following programming and protocol recommendations are to assist the user when staining on Biocare's ONCORE Pro Automated Slide Staining System for research applications. The user is responsible for further optimizations of the protocol. The ONCORE Pro will apply reagent as required in the selected protocol. Refer to the instrument manual for detailed instructions on instrument operation and additional protocol options. Uncap the probe vials and place in the ONCORE Pro reagent tray.

OPPR0001 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: EBER Probe
Protocol Template (Description): CISH RNA Template 1
Reagent Name, Time, Temp.: EBER Probe, 30 min., 55ºC

Limitations:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

Precautions:

1. Refer to reagent Safety Data Sheet for precautions.
2. This product contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC.
Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976)[5]
3. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.[6]
4. Microbial contamination of reagents may result in an increase in nonspecific staining.
5. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
6. Do not use reagent after the expiration date printed on the vial.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

References:

1. Epstein M, Achong B, Barr Y. Morphological and biological studies on a virus in cultured lymphoblast from Burkitt's lymphoma. J Exp Med. 1965 May 1;121:761-70.
2. Henle G, Henle W, Clifford P, et al. Antibodies to EB virus in Burkitt's lymphoma and control groups J. Nat. Cancer Inst. 1969; 43:1147-1157.
3. Komano, J S. Maru, K. Kurozumi,t. Oda, K. Takada. Oncogenic role of Epstein-Barr virus encoded RNAs in Burkitt's lymphoma cell line Akata. J. Virol 1999 73:9827-31.
4. Jaffe ES, Diebold J, Harris NL, Muller-Hermelink HK, Flandrin G, Vardiman JW. Burkitt's lymphoma: a single disease with multiple variants. The World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. Blood. 1999 Feb 1; 93(3):1124.
5. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
6. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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