RISH™ Epstein-Barr (EBER)

Hybridization Probe
901-RPI0001-112117

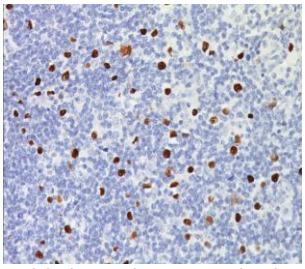
BIOCARE MEDICAL

Catalog Number: **RPI0001T**

Description: Approximately 20 tests at 20 microliters per test

Intended Use:
For In Vitro Diagnostic Use
This probe is used in the study of monoclonality in lymphoid tumors, lymphoproliferative syndromes, myelomas and for the study of immunodeficiency associated lymphoproliferative syndromes.
FOR DISTRIBUTION OUTSIDE THE UNITED STATES ONLY.

Summary & Explanation:
The Epstein-Barr virus 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). The *in situ* hybridization technique offers an important advantage over immunohistochemistry, as it virtually lacks background, and allows a clean and sharp viewing of the histological preparation.



Hodgkin's Lymphoma stained with RISH EBER probe

Principle of Procedure
This digoxigenin-labeled DNA probe will hybridize to its specific EBER RNA target in cells infected by the Epstein-Barr virus. The labeled probe is detected with an unconjugated anti-digoxigenin antibody, followed by a polymerized HRP incubation step. The DNA probe is indirectly evidenced by a colorimetric reaction.
Known Applications:
in situ hybridization (formalin-fixed paraffin-embedded tissues)
Supplied As:
RTU DNA probe in hybridization buffer
Materials and Reagents Needed But Not Provided:
RISH™ Detection Kit (RI0207KG or RI0213KG)*
Decloaking Chamber™ (pressure cooker)*
RISH™ Retrieval Solution (RI0209M)*
IQ Kinetic Slide Stainer* or other hybridization oven
IQ Aqua Sponge*
Positively charged microscope slides
Desert Chamber* (drying oven)
Positive and negative tissue controls
Xylene (could be substituted with xylene substitute)
Ethanol or reagent alcohol
Deionized or distilled water
TBS Wash Buffer (TWB945)*
Hematoxylin*

Materials and Reagents Needed But Not Provided Cont'd:
Bluing reagent*
Mounting medium*
Peroxidase*
HybriSlip™ (or equivalent)*
Thermal Test Strips

* Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.
Species Reactivity: EBER RNA in reactive and tumoral cells.
Cellular Localization: Nuclear
Storage and Stability:
Store probe at 2ºC to 8ºC. Do not use after expiration date printed on vials. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.
Protocol Recommendations:
Note: Biocare offers two detection kits for individual RISH probes. Biocare Kit RI027KG utilizes HRP / DAB and Kit RI0213 utilizes an AP/ Warp Red end-point.
1. Deparaffinization
a. Deparaffinize slides as per standard procedures.
b. Perform 5 minute hydrogen peroxide block.
c. Wash with distilled water, and place onto IQ Stainer at room temperature (RT).
2. Protein Digestion/ Retrieval
The following recommendations should be used as a starting point for tissues fixed 24 hours or longer. Tissues fixed less than 24 hours may require a further dilution of RISHzyme to buffer and/ or a heat pretreatment at lower temperature.
a. Prepare digestion reagent by combining 1 part enzyme to 1 part buffer. If tissues appear over-digested, consider: 1:3, 1:4 or 1:5 digestion reagent to buffer. (Working solution is stable for 7 days at 4ºC).
b. Place 200 µl onto tissue sample for 1 minute at RT.
c. Wash twice with distilled water, 2 minutes each wash.
d. Retrieve section with RISH Retrieval using Biocare's Decloaking Chamber*, followed by a wash in distilled water.
i. Suggested parameters: 90°C for 15 minutes, followed by 10 minute cool down in RISH Retrieval. If tissues appear over-digested, retrieve sections at 80°C or lower for 15 minutes followed by 10 minute cool down in RISH Retrieval.
ii. Wash in distilled water
iii. See technical notes if using a water bath or hotplate
3. Probe Hybridization
a. Use a Kimwipe to wipe off excess water around tissue section.
b. Apply 20 µl of RISH probe onto tissue section and cover slip with 22×22mm cover slip.
c. Place slides onto a preheated IQ Kinetic Slide Stainer** or humidity chamber at 37°C (DNA targeting probes) for 60 minutes or 55°C (mRNA targeting probes) for 30-60 minutes.
Note: **RISH DNA Targeting Probes (CMV, etc)** require denaturation at 95°C for 5 minutes prior to the 60 minute hybridization at 37°C.
4. Post-Hybridization Washing
a. Remove slides from incubation and put directly into TBS at RT. Briefly agitate until cover slip comes off.
b. Wash 5 minutes in TBS wash buffer at 55°C. Then, place slides in TBS wash buffer at RT for 5 minutes. Slight agitation in buffers and stringency wash is highly recommended.
5. Detection of Probe
a. Remove slides from TBS and use a Kimwipe to wipe around the edges of tissue. Apply PAP Pen, if necessary.
b. Place slides onto RT IQ Stainer or slide rack.
c. Decant TBS, and put 4 drops of RISH Secondary Reagent onto tissue sample, and incubate for 15 minutes.

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5. Detection of Probe Cont'd

d. Wash with TBS twice, 2 minutes each.
e. Decant TBS. Add 4 drops RISH HRP Tertiary Reagent or RISH AP Tertiary Reagent onto tissue sample, and incubate for 15 minutes.
f. Wash with TBS twice, 2 minutes each.
g. Decant TBS. Apply 4 drops of prepared Betazoid DAB to tissue samples if RISH HRP Tertiary Reagent was used in previous step. Use Warp Red if RISH AP Tertiary Reagent was used. Incubate for 5 minutes (apply 1 drop chromogen to 1 ml of buffer).
h. Wash with distilled water and examine slides on microscope prior to counterstaining.

6. Counterstaining

a. Briefly soak slides in CAT Hematoxylin for 5-6 seconds. Immediately rinse with distilled water. Excessive counterstaining will obscure specific signal. Reduce time in hematoxylin if too dark.
b. Soak slides in Tacha's Bluing solution for 5-6 seconds, and rinse with distilled water.

7. Optional: DAB Sparkle may be applied to sections to enhance DAB contrast. Apply 2-3 drops of DAB Sparkle directly to sections and incubate for 30 seconds to 1 minute. Wash in distilled water.

8. Cover Slipping

a. Dehydrate through graded alcohols and finish in xylene. If Warp Red chromogen is used, briefly dehydrate (30 seconds) through graded alcohols and xylene.
b. Apply 1 drop of mounting media to an appropriate cover slip and mount.
c. Allow to dry.

Technical Notes:

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.

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.

If using commercially available humidity chambers, hybridize probe for 30-60 minutes. Both incubator and humidity chamber must be at 55 ºC when hybridizing probe. Other hybridization chambers can be used, but measures should be taken to ensure that chamber is hermetically sealed during hybridization.

*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.

**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.

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.

Limitations:

The optimum parameters and protocols for a specific application can vary. These include, but are not limited to fixation, heat retrieval or enzymatic digestion, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended hybridization and incubation times listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Precautions:

This hybridization probe contains substances in low concentrations and volumes that are harmful to health. Avoid any direct contact with reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments).

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011

Troubleshooting:

Follow the reagent specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

Troubleshooting Guide:

No Staining

1. Critical reagent (such as probe) omitted.
2. Incorrect denaturation / hybridization temperature (less than 95°C / 37°C) used.
3. Staining steps performed incorrectly or in the wrong order.
4. Low or compromised target DNA / RNA.
5. Detection reagent incubations too short.
6. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining

1. Tissue is either over-fixed or under fixed.
2. Denaturation / hybridization temperatures incorrect.
3. Probe incubation time too short.
4. Low expression of RNA, contamination of tissues with RNases or RNA degradation.
5. Compromised genomic or target DNA.
6. Over-development of substrate.
7. Omission of critical reagent (digestion or retrieval solution).
8. Incorrect procedure in reagent preparation.
9. Improper procedure in steps.
10. Incorrect hybridization temperature (greater than 37ºC) used.

Non-specific or High Background Staining

1. Variable fixation time.
2. Substrate is overly developed.
3. Tissue was inadequately rinsed.
4. Deparaffinization incomplete.
5. Tissue damaged or necrotic.
6. Sections dried during hybridization.

Tissues Falling off Slide

1. Slides were not positively charged.
2. A slide adhesive was used in water bath.
3. Tissue was not dried properly.
4. Tissue contained too much fat.
5. Tissue may be over digested.

Specific Staining too Dark

1. Incubation of probe, secondary or tertiary too long.

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; 121:761-70.
2. Henle G, *et al.* Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. J Nat Cancer Inst. 1969; 43:1147-57.
3. Komano J, *et al.* Oncogenic role of Epstein-Barr virus encoded RNAs in Burkitt's lymphoma cell line Akata. J Virol. 1999 73:9827-31.
4. Jaffe ES, *et al.* 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. 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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