**RISH™ Lambda Light Chain DNA Probe**

**Hybridization Probe**

**Control Number:** 901-RPI0005-082814

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**Catalog Number:** RPI0005T  
**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.

**Summary & Explanation:**  
Lambda mRNA may be detected in normal and neoplastic B-cells in human lymphoid tissue. Restriction of either Kappa or Lambda mRNA denotes monoclonality of lymphoid neoplasms and is useful in distinguishing between neoplastic and reactive lymphoid proliferations. 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. It is also useful to differentiate cells that have absorbed immunoglobulins, and are therefore detectable by immunohistochemistry, but in fact do not produce immunoglobulin, as occurs with the Reed-Sternberg cells of Hodgkin’s disease.

Bone marrow myeloma of neck stained with Lambda RISH probe

**Principle of Procedure:**  
This DNA probe will hybridize to its specific mRNA target in tissues. 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 colored reaction.

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

**Supplied As:**  
RTU DNA probe in hybridization buffer

**Materials and Reagents Needed But Not Provided:**  
- Hybridization Probe
- RISH DNA Targeting Probes (CMV, etc)
- BIOTINylated DNA Targeting Probes
- DIG-labeled DNA Targeting Probes
- Thermal Test Strips
- Hybridization Chamber
- IQ Kinetic Slide Stainer
- Bio-care’s Decloaking Chamber
- Thermo-slip
- RISH HRP Tertiary Reagent or RISH AP Tertiary Reagent
- RISH Secondary Reagent
- RISH Tertiary Reagent
- 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
- Bluing Reagent
- Mounting medium
- Peroxidase

**Protocol Recommendations:**

1. **Deparaaffinization**
   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**
   - RISH™ Detection Kit (RI0207KG or RI0213KG)
   - 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
   - Bluing Reagent
   - Mounting medium
   - Peroxidase

3. **Probe Hybridization**
   a. Use 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.

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.
   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...
Protocol Recommendations cont’d:
Reagent was used. Incubate for 5 minutes (apply 1 drop chromogen to 1 ml of buffer).
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.
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.

Technical Notes:
This test should be performed on tissue sections where the presence of Lambda Light Chain mRNA 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 °C 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.

Performance Characteristics:
The optimum parameters and protocols for a specific application can vary. These include, but are not limited to: fixation, heat reaction 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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

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:

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 37°C) 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 glass slide 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.

Limitations & Warranty:
There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

References: