

# RISH™ Kappa Light Chain DNA Probe

Hybridization Probe  
902-RI0004-112217

**BIOCARE**  
M E D I C A L

**Catalog Number:** RI0004T  
**Description:** Approximately 20 tests at  
20 microliters per test

## Intended Use:

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

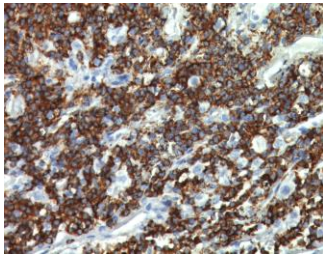
## Summary & Explanation:

Kappa mRNA may be detected in normal and neoplastic B-cells in human lymphoid tissue. Restriction of kappa 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.

## 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 colorimetric reaction.



Bone marrow plasma cell myeloma stained with RISH Kappa probe

## 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\*

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:** Human Kappa Light Chain RNA

**Cellular Localization:** Cytoplasmic

## Storage and Stability:

Store probe at 2°C to 8°C. 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 2°C to 8°C.

## Protocol Recommendations:

Refer to RISH™ Detection Kit (RI0207KG or RI0213KG) data sheet for specific protocol recommendations.

## Technical Notes:

This test should be performed on tissue sections where the presence of Kappa 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.

## 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.



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## Precautions:

This hybridization probe contains formamide in 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).

## Technical Support:

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

## 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 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. Beck RC, *et al.* Automated colorimetric *in situ* hybridization (CISH) detection of immunoglobulin (Ig) light chain mRNA expression in plasma cell (PC) dyscrasias and non-Hodgkin lymphoma. *Diagn Mol Pathol.* 2003 Mar; 12(1):14-20.
2. Shaw GR. Nonsecretory plasma cell myeloma--becoming even more rare with serum free light-chain assay: a brief review. *Arch Pathol Lab Med.* 2006 Aug; 130 (8):1212-5.
3. Lee LH, Cioc A, Nuovo GJ. Determination of light chain restriction in fine-needle aspiration-type Immunohistochem *Mol Morphol.* 2004 Sep; 12(3):252-8.
4. Stewart CJ, *et al.* Immunoglobulin light chain mRNA detected by *in situ* hybridisation in diagnostic fine needle aspiration cytology specimens. *J Clin Pathol.* 1996 Sep; 49(9):749-54.
5. Wilkens L, *et al.* Microwave pretreatment improves RNA-ISH in various formalin-fixed tissues using a uniform protocol. *Pathol Res Pract.* 1996 Jun; 192(6):588-94.
6. Peter J. Delves and Ivan M. Roitt. Immunoglobulin genes. *Encyclopedia of Immunology.* Pag1323. Second edition. Academic Press Limited (1988)
7. Weiss LM, *et al.* Detection of immunoglobulin light-chain mRNA in lymphoid tissues using a practical *in situ* hybridization method. *Am J Pathol* 1990 Oct; 137 (4):979-88.
8. Ruprai AK, *et al.* Localization of immunoglobulin light chain mRNA expression in Hodgkin's disease by *in situ* hybridization. *J Pathol.* 1991 May; 164(1):37-40.
9. 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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