RISH™ Kappa Light Chain DNA Probe

Hybridization Probe

ISO 9001&13485 CERTIFIED

Control Number: 902-RI0004-010512

Catalog Number: RI0004T

Description: Approximately 20 tests at 20 microliters per test

Intended Use:

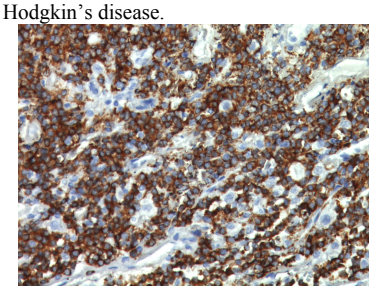
For Research use only. Not for use in diagnostic procedures.

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:

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.



Bone marrow plasma cell myeloma stained with RISH kappa 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 colormetric 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:

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*

Material and Reagents Needed But Not Provided cont'd:

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 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:

Refer to RISH™ Detection Kit (RI0207KG or RI0213KG) datasheet 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 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.

Performance Characteristics:

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers 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.

BIOCARE MEDICAL, LLC.
4040 Pike Lane
Concord, CA 94520 USA

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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

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).

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

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:

1. Beck RC, Tubbs RR, Hussein M, Pettay J, Hsi ED. 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, Farquharson MA, Kerr T, McCorriston J. 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, von Wasielewski R, Werner M, Nolte M, Georgii A. 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, Movahed LA, Chen YY, Shin SS, Stroup RM, Bui N, Estress P, Bindl JM. 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, Pringle JH, Angel CA, Kind CN, Lauder I. 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-Third Edition CLSI document M29-A3 Wayne, PA 2005