RISH™ Dual Kappa / Lambda Probe

Hybridization Probe Cocktail

Control Number: 902-RI0027-060115

Catalog Number: RI0027T

Description: Approximately 20 tests at 20 microliters per test

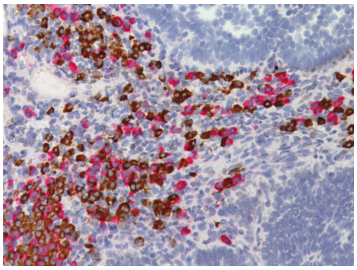
Intended Use:

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 and / or Lambda light chain 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 Dual Kappa / Lambda Probe cocktail can simultaneously detect polyclonal plasma cell infiltrates in a single tissue section.

The technique of *in situ* hybridization 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.



Polyclonal plasma cells surrounding nests of basal cell carcinoma. Section stained with RISH™ Dual probe cocktail.

Principle of Procedure

This DNA probe cocktail (digoxigenin labeled Kappa + biotinylated Lambda) will hybridize to its specific mRNA target in tissue sections. The labeled probes are simultaneously detected with a cocktail of unconjugated anti-digoxigenin + anti-biotin antibodies, followed by a double-stain detection of polymerized anti-mouse-HRP and anti-rabbit ALP. The DNA probes are indirectly evidenced by successive colorimetric reactions (Kappa / brown & Lambda/ red).

Known Applications:

in situ hybridization on formalin-fixed paraffin-embedded (FFPE) tissues.

Supplied As:

RTU DNA probes in hybridization buffer.

Materials and Reagents Needed But Not Provided:

RISH™ Kappa / Lambda Dual Detection Kit (RI0208KG)
Decloaking Chamber™ (pressure cooker)*
RISH™ Retrieval Solution (RI0209M)*
IQ Kinetic Slide Stainer* or other hybridization oven
IQ Aqua Sponge*
Microscope slides, positively charged
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*

Material 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:

Human Kappa / Lambda Light Chain mRNA

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.

Staining Protocol Recommendations:

Refer to RISH™ Dual Detection Kit (RI0208KG) data sheet for specific protocol recommendations.

Technical Notes:

This test should be performed on tissue sections where the presence of Kappa or 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 cocktailed-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:

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:

1. This product contains less than 0.1% sodium azide. Exposure to sodium azide may be harmful. 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 (9).
2. 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. Avoid contacting the skin and mucous membranes with reagents and specimens, and follow standard laboratory precautions to prevent exposure to eyes and skin. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (10)
3. Microbial contamination of reagents may result in an increase in innacurate results.
4. Do not use reagent after the expiration date printed on the vial.
5. The SDS is available upon request and is located at http://biocare.net.

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 hybridization temperature (less than 55°C) used
3. Staining steps performed incorrectly or in the wrong order
4. Low or compromised target 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. Probe incubation time too short
3. Low expression of RNA, contamination of tissues with RNases or RNA degradation
4. Over-development of substrate
5. Omission of critical reagent (digestion or retrieval solution)
6. Incorrect procedure in reagent preparation
7. Improper procedure in steps
8. Incorrect hybridization temperature (greater than 55ºC) 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 preparations of B-cell lymphomas by mRNA in situ hybridization. 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. Delves PJ, Roitt IM. Immunoglobulin genes. Encyclopedia of Immunology. 2nd ed. Academic Press Limited, 1988, p 1323.
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. 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."
10. 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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