RISH™ Kappa Light Chain DNA Probe

Hybridization Probe
902-RI0004-110822

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Predilute	RI 0004 T	20 tests	Ready-to-use	N/A
ONCORE Pro	OPPR 0004 T60	60 tests	Ready-to-use	N/A

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.

Species Reactivity:
Human Kappa Light Chain RNA

Cellular Localization: Cytoplasmic

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

Supplied As:
RI0004
(20 tests at approximately 20 µL per test) is provided in hybridization buffer containing dextran sulfate and nucleic acid carriers.

OPPR0004
(60 tests at approximately 130 µL per test) is provided in hybridization buffer containing dextran sulfate and nucleic acid carriers.

Reconstitution, Dilution and Mixing:
Products are supplied as ready-to-use DNA probe in hybridization Buffer. No reconstitution, dilution or mixing required.

Storage and Stability:
Store probe at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Materials and Reagents Required but Not Provided:
Microscope slides, positively charged
Desert Chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
ISH Dewax Kit (OPRI6020K)*
Decloaking Chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*(TBS)
RISH™ Retrieval Solution (RI0209M) *
ISH Retrieval (AR3) (OPRI6021) *
ISHzyme Kit (OPRI6039K)*
Peroxidase block*
Probe Enhancer (RNA) (OPRI6024) *
Negative control reagents*

Materials and Reagents Required but Not Provided Cont'd:
ISH Detection Reagents*
Chromogens*
Hematoxylin*
Bluing reagent*
Mounting medium*
IQ Aqua Sponge*
HybriSlip™ (or equivalent) *
IQ Kinetic Slide Stainer* or other hybridization oven
ONCORE Pro Automated Slide Stainer*

* Refer to the Biocare Medical website located at http://biocare.net for information regarding catalog numbers and ordering. Certain reagents listed above are based on specific application and detection system used.

Staining Protocol Recommendations (Manual Use):
Refer to RISH™ Detection Kit (RI0207KG or RI0213KG) datasheet for specific protocol recommendations.

Technical Notes:
1. 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.
2. 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.
3. If using commercially available humidity chambers, hybridize probe for 30-60 minutes. Both incubator and humidity chamber must be at 55ºC when hybridizing probe. Other hybridization chambers can be used, but measures should be taken to ensure that chamber is hermetically sealed during hybridization.
4. 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.
5. 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.
6. 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.

Staining Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):
The following programming and protocol recommendations are to assist the user when staining on Biocare's ONCORE Pro Automated Slide Staining System for research applications. The user is responsible for further optimizations of the protocol. The ONCORE Pro will apply reagent as required in the selected protocol. Refer to the instrument manual for detailed instructions on instrument operation and additional protocol options. Uncap the probe vials and place in the ONCORE Pro reagent tray.

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Staining Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System) Cont'd:

OPPR0004 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: Kappa Probe
Protocol Template (Description): CISH RNA Template 1
Reagent Name, Time, Temp.: Kappa Probe, 30 min., 55°C

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.

Precautions:

1. Refer to reagent Safety Data Sheet for precautions.
2. This product contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC.
Sodium azide (NaN_3) used as a preservative is toxic if ingested. 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. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976)[8]
3. 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. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.[9]
4. Microbial contamination of reagents may result in an increase in nonspecific staining.
5. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
6. Do not use reagent after the expiration date printed on the vial.

Technical Support:

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

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.

References Cont'd:

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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