

RISH™ DNA Positive Control Probe

Hybridization Probe

Control Number: 902-4026-091117

Catalog Number: BRR4026 T

Description: Approximately 20 tests at 20 microliters

Intended Use:

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

The DNA positive control probe is intended to be used to assess the integrity of DNA in formalin-fixed paraffin-embedded (FFPE) tissues by *in situ* hybridization.

Summary & Explanation:

This digoxigenin-labeled hybridization probe recognizes Alu repetitive sequences present within the mammalian genome (1-2). Specific hybridization of this probe to human Alu in FFPE tissues indicates that the test material contains intact DNA. This probe is to be used as a control when running RISH™ specific DNA targeting probes within Biocare's product line. Weak or light staining in a test sample indicates that specifically targeted DNA may be compromised.

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.

Principle of Procedure:

This digoxigenin-labeled DNA probe will hybridize specifically to Alu repetitive sequences within the human genome. The labeled probe is detected with an unconjugated anti-digoxigenin antibody, followed by a polymerized HRP or Alkaline phosphatase (AP) incubation step. The labeled probe is indirectly evidenced by a colorimetric reaction.

Known Applications:

in situ hybridization (formalin-fixed paraffin-embedded tissues)

Supplied As:

RTU DNA probe in buffered formamide with nucleic acid carriers

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

Species Reactivity: Human Alu specific sequences

Cellular Localization: Nuclear

Storage and Stability:

Store probe at 2°C to 8°C. Do not use after expiration date printed on vial. Avoid exposure to direct sunlight. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Staining Protocol Recommendations:

Refer to RISH™ Detection Kit (RI0207KG or RI0213KG)

Technical Notes:

This test should be performed on tissue sections where the presence of intact nuclear DNA is anticipated. 4-5 micrometer (µm) sections are sufficient to conduct the 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 Denaturation (95°C), hybridization (37°C) and post-hybridization detection steps. Detection steps can also be programmed on an automated staining system.

If using IQ1000 (single hot bar) place slides onto rack and denature on hot bar at 95°C for 5 minutes. After denaturation, remove rack and place on bench. Turn off hot bar and unplug unit. Cool hot bar (3-5 minutes) with running tap water until bar approximates 35-40°C. Re-set hot bar to hybridization temperature (37°C). Place water-saturated IQ Aqua sponge and a thermometer onto hot bar before hybridization. Check the temperature on the hot bar. It should not be higher than 40°C. Place rack with slides onto sponge, cover unit and incubate for 1 hour.

If an IQ Kinetic Slide Stainer is not available, consider using a hot plate to denature probe / slide at 95°C for 5 minutes. After denaturing slide, use a commercially available humidity chamber for hybridization at 60 min. Both incubator and humidity chamber must be at 37°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, allow to cool, and wash in distilled water prior to detection steps.

**The IQ Kinetic Slide 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 37°C for hybridization. Use the clear plastic hood to contain heat and moisture.

If probe appears cloudy, briefly vortex and heat to hybridization temperature (37°C) before application. 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.

Precautions:

1. This hybridization probe contains formamide in concentrations and volumes that are harmful to health. Avoid any direct contact with reagents. Take appropriate protective measures (wear disposable gloves, protective glasses, and lab garments).
2. The SDS is available upon request and is located at <http://biocare.net>.



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Technical Support:

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

References:

1. Weber AD, *et al.* Determining the origin of cells in tissue engineered skin substitutes: a pilot study employing in situ hybridization. *Pediatr Surg Int.* 2011 Mar;27(3):255-61.
2. Warncke B, *et al.* Experimental rat model for therapeutic retinal pigment epithelium transplantation--unequivocal microscopic identification of human donor cells by in situ hybridisation of human-specific Alu sequences. *Virchows Arch.* 2004 Jan;444(1):74-81.
3. 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."
4. 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.