RISH™ RNA Positive Control Probe

Hybridization Probe
902-6057-091117

BIOCARE MEDICAL

Catalog Number: BRR6057 T

Description: Approximately 20 tests at 20 microliters per test

Dilution: Ready-to-use

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

Summary & Explanation:
This digoxigenin-labeled oligonucleotide probe recognizes human 28S ribosomal RNA within tissue sections (1,2). This probe can be used as a control when running specific RNA targeting probes. It should be used to assess RNA integrity in FFPE tissue sections. Weak or light staining in a test sample indicates that specifically targeted mRNA 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 28S rRNA probe will hybridize specifically to mRNAs in tissue sections. 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 digoxigenin-labeled 28S rRNA 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

Species Reactivity: 28S ribosomal RNA

Cellular Localization: Cytoplasmic

Storage and Stability:
Store probe at 2ºC to 8ºC. Do not use after expiration date printed on vial. 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 RNA positive control probe should be used with RISH™ specific RNA probes (i.e. EBER, RNA negative control) in order to assess the presence of intact mRNA in test samples. The test should be performed on tissue sections where the presence of intact cytoplasmic RNA 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 probe has been standardized using Biocare's IQ Kinetic Slide Stainer for hybridization (55°C) and post-hybridization detection steps. Detection steps can also be programmed on an automated staining system.

If using IQ1000 (single hot bar) set hot bar to hybridization temperature (55°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 60°C. Place rack with slides onto sponge, cover unit and incubate for 30 to 60 minutes.

If an IQ Kinetic slide stainer is not available, consider using a commercially available humidity chamber for hybridization at 60 min. 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, 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 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. The use of probe in amounts less than recommended may lead to inconsistent results.

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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 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. (4)
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. (5)
3. Microbial contamination of reagents may result in inaccurate results.
4. 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.

References:
1. Lee D, Xiong S, Xiong WC. General introduction to in situ hybridization protocol using nonradioactively labeled probes to detect mRNAs on tissue sections. Methods Mol Biol. 2013;1018:165-74.
2. Paillasson S, *et al*. In situ hybridization in living cells: detection of RNA molecules. Exp Cell Res. 1997 25:231(1):226-33.
3. Wilkinson DG. In Situ Hybridization, A Practical Approach, Oxford University Press (1992) ISBN 0 19 963327 4.
4. 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."
5. 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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