RISH HRP Detection Kit

ISH Detection
901-RI0207-032323

BIOCARE MEDICAL

Available Product Formats	
Catalog Number	Volume
RI0207KG	6.0 mL

Intended Use:
For *in vitro* Diagnostic Use
The RISH™ HRP Detection Kit is intended for laboratory professional use in the pretreatment of formalin-fixed, paraffin-embedded (FFPE) tissues and the detection of digoxigenin labeled probes on FFPE tissues in an *in situ* hybridization (ISH) procedure performed manually or on Biocare Medical's ONCORE Pro Automated Slide Stainer and visualized by light microscopy. The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
The RISH™ HRP Detection Kit is to be used for the revealing of RISH probes. Under optimal conditions, RISH probes specifically hybridize to cognate mRNAs or DNA in formalin-fixed paraffin-embedded (FFPE) tissues. The Biocare RISH HRP Detection Kit provides reagents and materials for the preparation, pretreatment, hybridization, and detection of digoxigenin labeled probes.

Principle of Procedure:
This RISH™ HRP Detection Kit may be used in *in situ* hybridization (ISH) testing of formalin-fixed, paraffin-embedded tissue sections. Chromogenic *in situ* hybridization (CISH) permits the visual identification of specific mRNA or DNA nucleic acid sequences in tissues. Following pretreatment of the FFPE tissue and the application of the probe, the presence of a target nucleic acid is visualized by the sequential application of a secondary reagent that binds the digoxigenin labeled probe, followed by a tertiary enzyme antibody conjugate, and a chromogen reagent, to produce a colored reaction product that is visible by light microscopy.

Materials and Methods:
Reagents Provided:

Kit Catalog No.	Component Catalog No.	Component Description	Quantity x Volume
RI0207KG	RI0200G	RISHzyme™	1 x 6.0 mL
	RI0201G	RISHzyme™ Buffer	1 x 6.0 mL
	RI0203G	RISH™ Secondary Reagent	1 x 6.0 mL
	RI0204G	RISH™ HRP Tertiary Reagent	1 x 6.0 mL
	BDB900E	Betazoid DAB Chromogen	1 x 0.25 mL
	DS900G	Betazoid DAB Buffer	1 x 6.0 mL
	DS830G	DAB Sparkle	1 x 6.0 mL
	RMVL103	Mixing Vial	1 vial

Reconstitution, Mixing, Dilution, Titration:
The RISH™ HRP Detection Kit reagent(s) RISH™ Secondary Reagent, RISH™ HRP Tertiary Reagent, and DAB Sparkle are optimized and ready to use with Biocare ISH probes and ancillary reagents. No reconstitution, mixing, dilution, or titration is required.

The RISHzyme™ and RISHzyme™ Buffer are optimized for use with Biocare ISH probes and ancillary reagents. Mix 1-part concentrated RISHzyme™ to 1-parts RISHzyme™ Buffer (1:2 dilution).

The Betazoid DAB Chromogen and Betazoid DAB Buffer are optimized for use with Biocare ISH probes and ancillary reagents. Mix 1 drop chromogen to 1 mL of buffer.

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

Species Reactivity:
Digoxigenin-labeled probes on human tissues.

Supplied As:
RISHzyme – RI0200
Acidic enzyme solution, pH 3.1 – 3.3, and less than 0.1% sodium azide preservative. See Safety Data Sheet for additional details.

RISHzyme Buffer – RI0201
Buffered tris solution, pH 9.4 – 9.6, and less than 0.1% sodium azide preservative. See Safety Data Sheet for additional details.

RISH™ Secondary Reagent – RI0203
Buffered saline solution, containing a protein carrier and less than 0.01% ProClin 300 and/or less than 0.5% ProClin 950 as a preservative. See Safety Data Sheet for additional details.

RISH™ HRP Tertiary Reagent – RI0204
Buffered saline solution, pH 7.5-7.8, contains a protein carrier and less than 0.01% ProClin 300 and/or less than 0.5% ProClin 950 as a preservative. See Safety Data Sheet for additional details.

Betazoid DAB Chromogen – BDB900
DAB solution. See Safety Data Sheet for additional details.

Betazoid DAB Buffer – DS900
Buffered solution contains 3% Hydrogen Peroxide solution. See Safety Data Sheet for additional details.

DAB Sparkle – DS830
0.5% Cupric Sulfate Pentahydrate solution. See Safety Data Sheet for additional details.

Materials and Reagents Required but Not Provided:
Microscope slides positively charged.
Positive and negative tissue controls
Desert Chamber* or similar Drying oven (optional)
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
Decloaking Chamber* or similar pressure cooker (optional)
Deionized or distilled water
Wash buffer* (TBS)
Pretreatment reagents* (optional)
Peroxidase block* (optional)
Protein block* (optional)
RISH Probe*
Negative control reagents*
Hematoxylin* (counterstain)
Bluing reagent*
Mounting medium*
Coverglass
Light Microscope (40-400X magnification)
IQ Kinetic Slide Stainer or other hybridization oven*
IQ Aqua Sponge*
HybriSlip™ (or equivalent) *
Thermal Test Strips*
* Biocare Medical Products: 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 applications and detection system used.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

TP v1 (02/02/2023)

EC REP EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

RISH HRP Detection Kit

ISH Detection
901-RI0207-032323

Storage and Stability:
Store at 2ºC to 8ºC. The products are stable to the expiration date printed on the vial label when stored under these conditions. Do not use after expiration date. Storage under any condition other than those specified must be verified. The RISH™ HRP Detection Kit reagents RISH™ Secondary Reagent, RISH™ HRP Tertiary Reagent, and DAB Sparkle are ready-to-use and should not be diluted. The stability of user diluted reagent has not been established by Biocare.

RISH™ HRP Detection Kit diluted reagents, as indicated in Reconstitution, Mixing, Dilution, Titration section, should be used as instructed. ISHzyme working solution is stable for 7 days at 4ºC. The stability of user diluted Betazoid DAB reagent has not been established by Biocare.

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the probe is suspected, contact Biocare's Technical Support at 1-800-542-2002 or via the technical support information provided on biocare.net.

Specimen Preparation:
Tissues fixed in formalin are suitable for use prior to paraffin embedding. Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades.[1,2]

Properly fixed and embedded tissues expressing the specified antigen target should be stored in a cool place. The Clinical Laboratory Improvement Act (CLIA) of 1988 requires in 42 CFR §493.1259(b) that "The laboratory must retain stained slides at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination."[3]

Treatment of Tissues Prior to Staining:
Perform Heat Induced Epitope Retrieval (HIER) per recommended protocol below. The routine use of HIER prior to Immunohistochemistry (IHC) and ISH has been shown to minimize inconsistency and standardize staining.[4,5,17,18]

Warning and Precautions:
1. DAB is known to be a suspected carcinogen.
2. Do not expose DAB components to strong light or direct sunlight
3. DAB may cause sensitization of skin. Avoid contact with skin and eyes.
4. Wear gloves and protective clothing and take reasonable precautions when handling as DAB is classified as a danger and may cause cancer and is suspected of causing genetic defects.
5. DAB Sparkle contains Cupric Sulfate Pentahydrate. Wear gloves and protective clothing and take reasonable precautions when handling as DAB Sparkle is classified as an irritant and may cause skin contact sensitization. Avoid contact with eyes, skin, and mucous membranes.
6. Kit reagent(s) contain 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)[6]
7. Kit reagents contain less than 0.05% ProClin 300 and/or less than 1% ProClin 950. Wear gloves and protective clothing and take reasonable precautions when handling as ProClin is classified as an irritant and may cause skin contact sensitization. Avoid contact with eyes, skin, and mucous membranes.
8. Handle materials of human or animal origin as potentially biohazardous and dispose of such materials with proper precautions. In the event of exposure, follow the health directives of the responsible authorities where used.[7,8]
9. 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 into contact with sensitive areas, wash with copious amounts of water.[9]
10. Microbial contamination of reagents may result in an increase in nonspecific staining.
11. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
12. Do not use reagent after the expiration date printed on the vial.
13. The RISH™ HRP Detection Kit reagent(s) are optimized and ready to use with Biocare ISH probes and ancillary reagents. Refer to the ISH probes and other ancillary reagent instructions for use for recommended protocols and conditions for use.
14. Follow local and/or state authority requirements for method of disposal.
15. The SDS is available upon request and is located at http.//biocare.net.
16. Report any serious incidents related to this device by contacting the local Biocare representative and the applicable competent authority of the Member State or country where the user is located.

This RISH™ HRP Detection Kit contains components classified as indicated in the table below in accordance with the Regulation (EC) No. 1272/2008

Hazard	Code	Hazard Statement
	H316 H317	Causes mild skin irritation. May cause an allergic skin reaction.
	H334 H341 H350	May cause an allergy or asthma symptoms or breathing difficulties if inhaled. Suspected of causing genetic defects. May cause cancer.
	H400 H402 H412	Very toxic to aquatic life. Harmful to aquatic life. Harmful to aquatic life with long lasting effects.

Instructions for Use:
The RISH™ HRP Detection Kit reagent(s) are optimized and ready to use with Biocare ISH probes and ancillary reagents. Refer to the ISH probe and other ancillary reagent instructions for conditions for use. Incubation times and temperatures will vary depending on the probe and test design.

When using an automated staining instrument, consult the specific instrument operator manual and instructions for use for operating parameters.

General procedural steps for performing ISH:
1. Deparaffinize slides as per standard procedures.
2. Perform 5-minute hydrogen peroxide block.
3. Wash with distilled water, and place onto IQ Stainer at room temperature (RT).

Protein Digestion/ Retrieval
4. Prepare digestion reagent (1:2) by combining 1 part enzyme to 1 part buffer. If tissues appear over-digested, consider: 1:3 - 1:5 digestion reagent to buffer. The following recommendations should be used as a starting point for tissues fixed 24 hours or longer. Tissues fixed less than 24 hours may require a further dilution of RISHzyme to buffer and/ or a heat pretreatment at lower temperature.
5. Place 200 µL onto tissue sample for 1 minute at RT.
6. Wash twice with distilled water, 2 minutes each wash.
7. Retrieve section with RISH Retrieval using Biocare's Decloaking Chamber*, followed by a wash in distilled water. Suggested parameters: 90°C for 15 minutes, followed by 10-minute cool down in RISH Retrieval. If tissues appear over-digested, retrieve sections at 80°C or lower for 15 minutes followed by 10-minute cool down in RISH Retrieval.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

TP v1 (02/02/2023)

EC REP EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

8. Wash in distilled water.
9. See technical notes if using a water bath or hotplate.

Probe Hybridization

10. Use Kimwipe to wipe off excess water around tissue section.
11. Apply 20 µL of RISH probe onto tissue section and cover slip with 22×22mm cover slip.
12. Place slides onto a preheated IQ Kinetic Slide Stainer or humidity chamber at 37°C (DNA targeting probes) for 60 minutes or 55°C (mRNA targeting probes) for 30-60 minutes. See Technical Note #1.

Post-Hybridization Washing

13. Remove slides from incubation and put directly into TBS at RT. Briefly agitate until cover slip comes off.
14. Wash 5 minutes in TBS wash buffer at 55°C. Then, place slides in TBS wash buffer at RT for 5 minutes. Slight agitation in buffers and stringency wash is highly recommended.

Detection of Probe

15. Remove slides from TBS and use a Kimwipe to wipe around the edges of tissue. Apply PAP Pen, if necessary.
16. Place slides onto RT IQ Stainer or slide rack.
17. Decant TBS and put 4 drops of RISH Secondary Reagent onto tissue sample and incubate for 15 minutes.
18. Wash with TBS twice, 2 minutes each.
19. Decant TBS. Add 4 drops RISH HRP Tertiary Reagent onto tissue sample and incubate for 15 minutes.
20. Wash with TBS twice, 2 minutes each.
21. Decant TBS. Apply 4 drops of prepared Betazoid DAB to tissue samples and incubate for 5 minutes (apply 1 drop chromogen to 1 ml of buffer).
22. Wash with distilled water and examine slides with a microscope prior to counterstaining.

Counterstaining

23. Briefly soak slides in CAT Hematoxylin for 5-6 seconds. Immediately rinse with distilled water. Excessive counterstaining will obscure specific signals. Reduce time in hematoxylin if it is too dark.
24. Soak slides in Tacha's Bluing solution for 5-6 seconds, and rinse with distilled water.

Optional: DAB Sparkle

25. DAB Sparkle may be applied to sections to enhance DAB contrast. Apply 2-3 drops of DAB Sparkle directly to sections and incubate for 30 seconds to 1 minute. Wash in distilled water.

Cover Slipping

26. Dehydrate through graded alcohols and finish in xylene.
27. Apply 1 drop of mounting media to an appropriate cover slip and mount.

Technical Notes:

1. RISH DNA Targeting Probes (CMV, etc.) require denaturation at 95°C for 5 minutes prior to the 60-minute hybridization at 37°C.
2. The Biocare RISH HRP Detection Kit has been developed to detect digoxigenin labeled RISH probes by in situ hybridization. Routinely processed (FFPE) tissues in which the presence of cognate nucleic acid target is anticipated can be used.
3. Biocare's IQ Kinetic Slide Stainer was used for hybridization and post hybridization detection steps of RISH probes. Detection steps can also be programmed on an automated staining system (see below). Commercially available hybridization chambers can be used, but measures should be taken to ensure that chamber is hermetically sealed during hybridization. Both incubator and humidity chamber must be at 55ºC (mRNA targeting probes) or at 37°C (DNA targeting probes) when hybridizing probe.
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 reached (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 (mRNA targeting probes) or 37°C (DNA targeting probes) for hybridization Use the clear plastic hood to contain heat and moisture.
6. If using the 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.
7. If probe appears cloudy, briefly vortex and heat to hybridization temperature (37°C or 55°C) before application. The use of probe in amounts less than recommended may lead to inconsistent results. Detection of RISH probes may be performed on Biocare's intelliPATH™ Automated Stainer. Contact Biocare's Technical Support Staff for protocol recommendations.

Bone Marrow Biopsies:

For optimal results, bone marrow biopsies should be fixed for 24 hours in 10% neutral buffered formalin (NBF) or zinc formalin prior to decalcification. Biocare recommends for preservation of RNA integrity that bone marrows are decalcified in a formic acid or 10% EDTA based solution.[19,20] However, there are many methods of fixation and decalcification used in the clinical laboratory. The table below represents parameters and tissues that have tested positive for Kappa / Lambda mRNA RISH probes in our laboratory. All decalcification methods, including those mentioned, should be empirically determined for optimal pretreatment parameters. Biocare's protocol recommendations for the ratio of enzyme to buffer (1:2) should be used as a starting point for tissues fixed 24 hours or longer. Tissues fixed less than 24 hours may require a dilution of RISHzyme to buffer of 1:4 and a heat pretreatment of 80°C for 15 minutes to prevent digestion artifacts. Check for completion of decalcification by in-house methods and process tissues into paraffin according to standard procedures.

Fixations	Decalcification	Digestion RISHzyme buffer	Bone marrow positive by RISH™
NBF	10% HNO3	1:2 – 1:4	Plasma cell myeloma, Thoracic myeloma
NBF + zinc	Formic Acid / formaldehyde (Formical-4)	1:2 – 1:4	Bone marrow clinically undefined
NBF	RDO	1:2 – 1:4	Bone marrow clinically undefined
NBF + zinc	RDO	1:2 – 1:4	Bone tumor plasma cell myeloma

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011[10]

Positive Tissue Control:

External positive control materials should be fresh specimens fixed, processed, and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are indicative of correctly prepared tissues and proper staining techniques. One positive external tissue control for each set of test conditions should be included in each staining run.

The tissues used for the external positive control materials should be selected from patient specimens with well-characterized low levels of the positive target activity that gives weak positive staining. The low level of positivity for external positive controls is designed so to ensure detection of subtle changes in the probe sensitivity from instability or problems with the ISH methodology. Commercially available tissue control slides or specimens processed differently from the patient sample(s) validate reagent performance only and do not verify tissue preparation.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, rather than as an aid in formulating a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control:
Use a negative tissue control fixed, processed, and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of the ISH probe for demonstration of the target RNA, and to provide an indication of specific background staining (false positive staining). Also, the variety of different cell types present in most tissue sections can be used by the laboratorian as internal negative control sites to verify the ISH's performance specifications. The types and sources of specimens that may be used for negative tissue controls are listed in the Performance Characteristics section.
If specific staining (false positive staining) occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Nonspecific Negative Reagent Control:
Use a nonspecific negative reagent control in place of the probe with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the RNA site. Ideally, a negative reagent control contains a probe produced and prepared (i.e., diluted to same concentration using same diluent) for use in the same way as the probe but exhibits no specific reactivity with human tissues in the same matrix/solution as the Biocare probe. Diluent alone may be used as a less desirable alternative to the previously described negative reagent controls. The incubation period for the negative reagent control should correspond to that of the probe.

When panels of several probes are used on serial sections, the negatively staining areas of one slide may serve as a negative/nonspecific binding background control for other probes. To differentiate endogenous enzyme activity or nonspecific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate-chromogen or enzyme complexes (PAP, avidin-biotin, streptavidin) and substrate-chromogen, respectively.

Assay Verification:
Prior to initial use of a probe or staining system in a diagnostic procedure, the user should verify the probe's specificity by testing it on a series of in-house tissues with known performance characteristics representing known positive and negative tissues. Refer to the quality control procedures previously outlined in this section of the product insert and to the quality control recommendations of the CAP Certification Program[11] for Immunohistochemistry and/or the NCCLS IHC guideline[12] and In Situ Hybridization (ISH) guidelines[17]. These quality control procedures should be repeated for each new probe lot, or whenever there is a change in assay parameters.

Troubleshooting:
For product assistance, contact Biocare's Technical Support at 1-800-542-2002.

Interpretation of Staining:
An ISH probe works in conjunction with ancillary reagents to produce a brown color reaction at the RNA sites localized by the probe. Diluent ancillary reagents assist with providing a pH buffered environment to facilitate probe binding in the probe-RNA specific staining reaction. Prior to interpretation of results, the staining of controls must be evaluated by a qualified pathologist. Negative controls are evaluated and compared to stained slides to ensure any staining observed is not a result of nonspecific interactions.

Positive Tissue Control:
The positive tissue control stained with indicated probe should be examined first to ascertain that all reagents are functioning properly. The appropriate staining of target cells (as indicated above) is indicative of positive reactivity. If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid.

The color of the reaction product may vary depending on substrate chromogens used. Refer to substrate package inserts for expected color reactions. Further, metachromasia may be observed in variations of the method of staining.[13]
When a counterstain is used, depending on the incubation length and potency of the counterstain used, counterstaining will result in a coloration of the cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.

Negative Tissue Control:
The negative tissue control should be examined after the positive tissue control to verify the specificity of the labeling of the target RNA by the probe. The absence of specific staining in the negative tissue control confirms the lack of probe cross reactivity to cells/cellular components. If specific staining (false positive staining) occurs in the negative external tissue control, results with the specimen should be considered invalid.

Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

Patient Tissue:
Examine patient specimens stained with indicated probe last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the RNA was not detected, not that the RNA was absent in the cells/tissue assayed. If necessary, use a panel of probes to identify false-negative reactions.

Refer to Summary and Explanation, Limitations, and Performance Characteristics for specific information regarding indicated probe immunoreactivity.

Limitations:

General Limitations:
1. For *in vitro* diagnostic (IVD) Use
2. This product is for professional use only: *in situ* hybridization is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the ISH slide; and interpretation of the staining results.
3. For use by physician prescription only. (Rx Only)
4. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, probe trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.[14]
5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology, and other histopathological criteria. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of ISH probes, reagents, and methods

to interpret all the steps used to prepare and interpret the final ISH preparation.
7. The optimum protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, probe dilution, tissue section thickness and detection kit used. Refer to the probe and other ancillary reagent instructions for use for recommended protocols and conditions for use. The data sheet recommendations and protocols, where available, are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.
8. This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry.
9. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[15]
10. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated due to biological variability of RNA expression in neoplasms, or other pathological tissues.[16] Contact Biocare's Technical Support at 1-800-542-2002, or via the technical support information provided on biocare.net, with documented unexpected reaction(s).
11. Normal/nonimmune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results.
12. False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudo peroxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g., liver, breast, brain, kidney) depending on the type of immunostain used.[14]
13. A negative result means that the RNA was not detected, not that the RNA was absent in the cells or tissue examined.

Product Specific Limitations:
No additional product specific limitations.

Performance Characteristics:
Staining was performed and evaluated using internally standardized protocols, across a range of normal and neoplastic tissue types during product development.

Reproducibility:
The reproducibility of Biocare's detection systems and system reagents is verified through a measurement of intermediate precision in which various reagent lots were tested over an extended period of time using various operators, analysts, reagent lots, tissue samples, and equipment. The staining obtained for each detection reagent evaluated was consistent and performed as expected.

Troubleshooting:
1. No staining of any slides – Check to determine appropriate positive control tissue, probe, and detection products have been used. Check for incomplete or improper wax removal or pretreatment.
2. Weak staining of all slides – Check to determine appropriate positive control tissue, probe, and detection products have been used.
3. Excessive background of all slides – There may be high levels of endogenous HRP activity converting chromogen to colored end product (use peroxidase block), or excess non-specific protein interaction (use a protein block, such as serum- or casein-based blocking solution).
4. Tissue sections wash off slides during incubation – Check slides to ensure they are positively charged.
5. Specific staining too dark – Check protocol to determine if proper probe titer was applied to slide, as well as proper incubation times for all reagents. Additionally, ensure the protocol has enough washing steps to remove excess reagents after incubation steps are completed.

References:
1. Kiernan JA. Histological and Histochemical Methods: Theory and Practice. New York: Pergamon Press 1981.
2. Sheehan DC and Hrapchak BB. Theory and Practice of Histotechnology. St. Louis: C.V. Mosby Co. 1980.
3. Clinical Laboratory Improvement Amendments of 1988: Final Rule, 57 FR 7163, February 28, 1992.
4. Shi S-R, Cote RJ, Taylor CR. J Histotechnol. 1999 Sep;22(3):177-92.
5. Taylor CR, et al. Biotech Histochem. 1996 Jan;71(5):263-70.
6. 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.
7. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
8. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
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.
10. 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
11. College of American Pathologists (CAP) Certification Program for Immunohistochemistry. Northfield IL. Http://www.cap.org (800) 323-4040.
12. O'Leary TJ, Edmonds P, Floyd AD, Mesa-Tejada R, Robinowitz M, Takes PA, Taylor CR. Quality assurance for immunocytochemistry; Proposed guideline. MM4-P. National Committee for Clinical Laboratory Standards (NCCLS). Wayne, PA. 1997;1-46.
13. Koretzik K, Lemain ET, Brandt I, and Moller P. Metachromasia of 3-amino-9-ethylcarbazole (AEC) and its prevention in Immunoperoxidase techniques. Histochemistry 1987; 86:471-478.
14. Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and its pitfalls. Lab Med 1983; 14:767.
15. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen: a possible source of error in immunohistochemistry. AmJ Clin Path 1980; 73:626.
16. Herman GE and Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech & Histochem 1991; 66:194.
17. Wilkinson DG. In Situ Hybridization: A Practical Approach (Practical Approach Series). 2nd Ed. Oxford: Oxford University Press, 1999.
18. Nuovo GJ. In Situ Molecular Pathology and Co-Expression Analyses. 1st Ed. San Diego: Academic Press, 2013.
19. 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.
20. Shibata Y, et al. Assessment of decalcifying protocols for the detection of specific RNA by non-radioactive in situ hybridization in calcified tissues. Histochem Cell Biol. 2000 Mar; 113(3):153-9.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE
TP v1 (02/02/2023)

EC REP EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080