RISH HRP Detection Kit

ISH Detection
902-BRR0207-032323

BIOCARE MEDICAL

Available Product Formats	
Catalog Number	Volume
BRR0207KG	6.0 mL

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

Background Information:
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.

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

Materials and Methods:
Reagents Provided:

Kit Catalog No.	Component Catalog No.	Component Description	Quantity x Volume
BRR0207KG	BRR0200G	RISHzyme™	1 x 6.0 mL
	BRR0201G	RISHzyme™ Buffer	1 x 6.0 mL
	BRR0203G	RISH™ Secondary Reagent	1 x 6.0 mL
	BRR0204G	RISH™ HRP Tertiary Reagent	1 x 6.0 mL
	BRR900AE	Betazoid DAB Chromogen	1 x 0.25 mL
	BRR900BG	Betazoid DAB Buffer	1 x 6.0 mL
	BRR830G	DAB Sparkle	1 x 6.0 mL
	RMVL103	Mixing Vial	1 vial

* Refer to the Biocare Medical website located at http://biocare.net for information regarding catalog numbers and ordering.

Supplied As:
RISHzyme – BRR0200
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 – BRR0201
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 – BRR0203
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 – BRR0204
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 – BRR900A
DAB solution. See Safety Data Sheet for additional details.

Betazoid DAB Buffer – BRR900B
Buffered solution contains 3% Hydrogen Peroxide solution. See Safety Data Sheet for additional details.

DAB Sparkle – BRR930
0.5% Cupric Sulfate Pentahydrate solution. See Safety Data Sheet for additional details.

Reconstitution, Dilution and Mixing:
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.

Species Reactivity:
Digoxigenin-labeled probes on human tissues.

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.

Staining Protocol Recommendations:
Below are programming and protocol recommendations to assist the user when staining manually and/or using one of Biocare's Automated Staining Platforms for research applications. The user is responsible for further optimizations of the protocol.
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.
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.

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Staining Protocol Recommendations Cont'd:

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 37ºC (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 the hot bar and unplug the unit. Cool hot bar (3-5 minutes) with

Technical Notes Cont'd:

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

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. 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 it 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)[1]

Precautions Cont'd:
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.[2,3]
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.[4]
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.

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.

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

References:
1. 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.
2. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
3. 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.
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.

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