

ISH HRP Detection Kit for Digoxigenin-Labeled DNA Probes

Chromogenic Detection of Digoxigenin-Labeled Probes

Control Number: 901-4050K-090717

Catalog Number: BRI 4050 KG

Description: 6ml (Approximately 40 tests)

Intended Use:

For In Vitro Diagnostic Use

ISH HRP Detection Kit for Digoxigenin-Labeled DNA Probes is intended for the detection of digoxigenin-labeled DNA probes as part of an *in situ* hybridization (ISH) procedure on formalin-fixed, paraffin-embedded (FFPE) human tissues. 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 & Explanation:

ISH HRP Detection Kit for Digoxigenin-Labeled DNA Probes is useful for the chromogenic visualization of digoxigenin-labeled DNA probes hybridized to cognate DNA target sequences in an ISH procedure. The digoxigenin-labeled probe is detected with an unconjugated mouse anti-digoxigenin antibody, followed by a goat anti-mouse HRP polymer conjugate. Visualization is achieved by a colorimetric reaction with a compatible chromogen, facilitated by HRP.

Known applications:

in situ hybridization on formalin-fixed paraffin-embedded (FFPE) tissues.

Supplied As:

1. Mouse anti-Digoxigenin (BRI4049G) 6 ml
2. MACH 2 Mouse HRP-Polymer (MHRP520G) 6 ml

Materials and Reagents Needed But Not Provided:

Decloaking Chamber™ (pressure cooker)*
IQ Kinetic Slide Stainer* or other hybridization oven
IQ Aqua Sponge*
Microscope slides, positively charged
Desert Chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
Deionized or distilled water
Peroxidase block*
Pronase kit*
Diva Decloaker*
Wash buffer*(TBS/PBS)
Digoxigenin-labeled Probes
SSC Wash Buffer*
Deep Space Black Chromogen kit* or DAB*
Hematoxylin*
Bluing Reagent*
Mounting medium*
HybriSlip™* (or equivalent)
Thermal test strips*

*Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Storage and Stability:

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

Protocol Recommendations:

DAY 1

1. Deparaffinization

- a. Deparaffinize slides in Xylene as per standard procedures.
- b. Perform 5 minute hydrogen peroxide block.
- c. Wash with distilled water, and place onto IQ Stainer at room temperature (RT).

2. Protein Digestion/ Retrieval

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 Pronase concentrate in buffer and/or a heat pretreatment at lower temperature.

- a. Prepare Pronase working solution by combining 1 part enzyme to 9 parts buffer. (Working solution is stable for 14 days at 4°C.)
- b. Place 4 drops onto tissue sample for 10 minutes at RT.
- c. Wash in distilled water.
- d. Preheat Diva Decloaker retrieval solution to 95°C in Biocare's Decloaking Chamber™. Retrieve section with Diva Decloaker using Biocare's Decloaking Chamber™, followed by a wash in distilled water.
- i. Suggested parameters: 95°C for 25 minutes, followed by 5 minute cool down in the retrieval solution.
- ii. Wash in distilled water.
- iii. Air dry slides lightly.

3. Probe Hybridization

- a. Ensure probe is thoroughly mixed by inverting several times or briefly vortexing.
- b. Apply 20 µl of digoxigenin-labeled probe onto tissue section and cover with 22×22mm cover slip.
- c. Denaturation step: Place slides onto an IQ Stainer or a hot plate at 95°C for 5 minutes.
- d. Hybridization step: Place slides onto an IQ Stainer (See Technical Notes) or humidity chamber at 40°C for 16-24 hours.

DAY 2

4. Post-Hybridization Washing

- a. Wash slides with SSC Wash Buffer on 40°C IQ Stainer. Wait 5-10 minutes until cover slips loosen. Carefully move coverslips sideways and remove.
- b. Flood the slides with SSC Wash Buffer and incubate for 5 minutes on 40°C IQ Stainer. Do not agitate. Do not let tissues dry out during post-hyb washing. (Post- hyb washing may also be done in 40°C water bath).
- c. Wash slides in RT TBS Wash Buffer.

5. Detection of Probe

- a. Decant TBS and add 4 drops of Mouse anti-Digoxigenin onto tissue sample. Incubate for 15 minutes.
- b. Wash with TBS buffer.
- c. Decant TBS and add 4 drops of MACH 2 Mouse HRP-Polymer onto tissue sample. Incubate for 15 minutes (see Technical Note 6.).
- d. Wash with TBS buffer.
- e. Decant TBS, apply 4 drops of Deep Space Black Chromogen working solution (prepared according to chromogen kit data sheet) onto tissue sample. Incubate for 5 minutes. DAB Chromogen can be used instead of Black Chromogen (prepare according to chromogen kit data sheet).
- f. Wash with distilled water.

6. Counterstaining

- a. Briefly soak slides in CAT Hematoxylin for 5-6 seconds. Immediately rinse with distilled water.
- b. Soak slides in Tacha's Bluing Solution for 5-6 seconds, and rinse with distilled water.

7. Cover Slipping

- a. Dehydrate through graded alcohols and finish in xylene.
- b. Apply 1 drop of mounting media to an appropriate cover slip and mount.
- c. Allow to dry.

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Technical Notes:

1. The Biocare ISH HRP Detection Kit for Digoxigenin-Labeled DNA Probes has been developed to detect digoxigenin-labeled probes at a concentration of 0.05-0.10 ng/ μ L by *in situ* hybridization. The use of probe in amounts less than recommended may lead to inconsistent results. Routinely processed (FFPE) tissues in which the presence of cognate nucleic acid target is anticipated can be used.
2. To reduce non-specific background, Background Punisher (BP974) may be used for 10 min after Mouse anti-Digoxigenin.
3. Biocare's IQ Kinetic Slide Stainer may be used for hybridization and post-hybridization detection steps of digoxigenin-labeled probes. Detection of digoxigenin-labeled probes may be performed on Biocare's intelliPATH™ Automated Slide Stainer with intelliPATH™ DAB Chromogen Kit (IPK5010).
4. 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 40°C for hybridization. Use the clear plastic hood to contain heat and moisture. Commercially available solutions for fog prevention may be used to prevent excessive accumulation of the moisture inside the clear plastic hood.
5. If using commercially available humidity chambers, hybridize probe for 16-24 hours. Both incubator and humidity chamber must be at 40°C when hybridizing probe. Other hybridization chambers can be used, but measures should be taken to ensure that chamber is hermetically sealed during hybridization.
6. If red chromogen detection is desired, use MACH 2 Mouse AP-Polymer (MALP521) with 15 minutes incubation time, followed by Warp Red™ Chromogen (WR806) with 7-10 minutes incubation time.

Limitations:

The optimum parameters and protocols for a specific application can vary. These include, but are not limited to fixation, enzymatic digestion, incubation times, tissue section thickness, and detection kit used. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. 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.

Quality Control:

Refer to 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

References:

1. Wilkinson DG. In Situ Hybridization: A Practical Approach (Practical Approach Series). 2nd Ed. Oxford: Oxford University Press, 1999.
2. Nuovo GJ. In Situ Molecular Pathology and Co-Expression Analyses. 1st Ed. San Diego: Academic Press, 2013.
3. 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.

Precautions

1. This product is not classified as hazardous. The preservative used in kit reagents is Proclin 950. The concentration of Proclin (less than 0.25%) does not meet the OSHA criteria for hazardous substances. Overexposure to Proclin can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. Wear disposable gloves when handling reagents.
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. 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. (3)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.
7. Consult OSHA, federal, state or local regulations for disposal of any toxic substances. Proclin™ is a trademark of Rohm and Haas Company, or of its subsidiaries or affiliates.

Troubleshooting:

Follow the reagent specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.