MM HRP Digoxigenin System

Mouse Antibodies on Mouse Tissues
902-4061K-052620

BIOCARE MEDICAL

Catalog Number: **BRR 4061K G**

Description: 6.0 mL

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

Summary & Explanation:
The MM HRP Digoxigenin System for use with mouse antibodies on mouse tissues is a detection system specifically formulated to prevent endogenous mouse IgG background staining when using a mouse primary antibody on mouse tissue. The MM HRP Digoxigenin System provides reagents to label the mouse primary antibody of interest with digoxigenin, even in the presence of other non-IgG proteins, and detect it on mouse tissue with an HRP-polymer system. With this detection system, monoclonal antibodies bound to mouse antigens in mouse tissue can be detected with high specificity and sensitivity, while significantly reducing or eliminating background. Even the most problematic tissues such as lymphatic, lung and kidney tissues are virtually background free. Additionally, the MM HRP Digoxigenin System does not require overnight incubation steps, allowing for rapid screening of multiple mouse primary antibody clones.

Known Applications:
Formalin-fixed paraffin-embedded (FFPE) tissues

Supplied As:
1. Mouse Linker (BRR4058B) 0.5mL
2. Mopping Reagent (MMMR611B) 0.5mL
3. Rabbit anti-Digoxigenin (BRR4059G) 6mL
4. MACH 2 Rabbit HRP-Polymer (RHRP520G) 6mL

Species Reactivity:
Mouse IgG heavy and light chains

Storage and Stability:
Store kit at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date.

Staining Protocol Recommendations:

Preparation of the Digoxigenin-labeled Antibody (1mL) (Figure 1):
1. Combine 80 µL of Mouse Linker with primary antibody (0.1 µg – 1.0 µg/mL concentration) and vortex for 5 seconds.
2. Incubate mixture at room temperature (RT) for 1 hour.
3. Add 40 µL of Mopping Reagent to mixture, vortex for 5 seconds, and incubate for 30 minutes at RT.
4. Add desired antibody diluent to a final volume of 1 mL.

The primary antibody is now labeled with digoxigenin and is ready to use. The antibody should be used within one to two days of preparation. Shelf life may vary and, ultimately, must be determined by the individual investigator.

Note: Instructions are for 1 mL of labeled antibody. Preparation can be scaled linearly (i.e. for 0.5 mL of labeled antibody, divide all reagent volumes by 2).

Staining Procedure:
1. Deparaffinize tissue sections in Slide Brite or xylene. Hydrate slides in a graded series of alcohol to water.
2. Apply Peroxidazed 1 for 5 minutes at RT.
3. Rinse slides in deionized (DI) water.
4. Pretreatment solution/protocol:

Heat-retrieval (optional): Heat slides in Biocare's Diva Decloaker, Reveal Decloaker, or Borg Decloaker using Biocare's Decloaking Chamber.
Proteolytic digestion (optional): Digest tissue with Trypsin or Pepsin.

5. Rinse slides with two changes of TBS wash buffer.
6. Protein block (optional): Apply Biocare's Background Punisher for 10 minutes at RT.

Staining Procedure Cont'd:
7. Rinse slides with TBS wash buffer.
8. Apply digoxigenin-labeled primary antibody to tissue and incubate for 30 minutes to 1 hour at RT.
9. Rinse slides with TBS wash buffer.
10. Apply Rabbit anti-Digoxigenin to tissue and incubate for 15 minutes at RT.
11. Rinse slides with TBS wash buffer.
12. Apply MACH 2 Rabbit HRP-Polymer to tissue and incubate at RT for 15 to 30 minutes.
13. Rinse slides with TBS wash buffer for 5 minutes.
14. Apply DAB for 5 minutes at RT. Wash in DI water.
15. Apply CAT Hematoxylin for 30 seconds to 1 minute or Tacha's Automated Hematoxylin for 5 minutes. Wash in DI water.
16. Apply Tacha's Bluing Solution for 1-2 minutes. Wash in DI water.
17. Dehydrate, clear and coverslip.

Technical Notes:
The optimal diluent is dependent on the primary antibody and must be determined by the investigator.

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 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. 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.

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

Troubleshooting Guide:

No Staining
1. Critical reagent (such as primary antibody) omitted.
2. Primary antibody not labeled
3. Staining steps performed incorrectly or in the wrong order.
4. Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, the wrong order or the wrong pretreatment.
5. Insufficient amount of antigen.

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Troubleshooting Guide Cont'd:

6. Primary antibody incubation period too short.
7. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining

1. Tissue is either over-fixed or under-fixed.
2. Primary antibody not labeled effectively
3. Primary antibody incubation too short.
4. Low expression of antigen.
5. Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment.
6. Over-development of substrate.
7. Excessive rinsing during wash steps.
8. Omission of critical reagent.
9. Incorrect procedure in reagent preparation.
10. Improper procedure in test steps.

Non-specific or High Background Staining

1. Tissue is either over-fixed or under-fixed.
2. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
3. Tissue may need a longer or a more specific protein block.
4. Substrate is overly-developed.
5. Tissue was inadequately rinsed.
6. Deparaffinization incomplete.
7. Tissue damaged or necrotic.

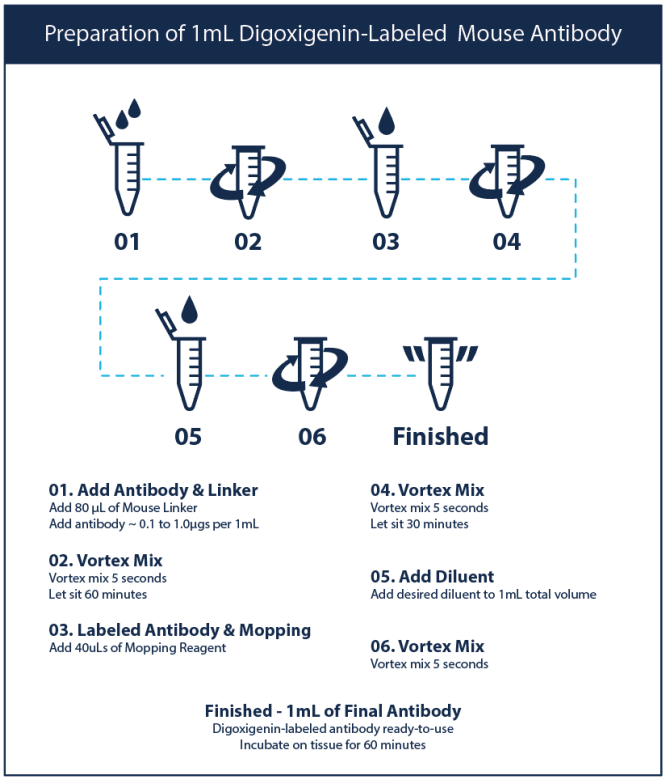
Tissues Falling Off

1. Slides were not positively charged.
2. A slide adhesive was used in the waterbath.
3. Tissue was not dried properly.
4. Tissue contained too much fat.

Specific Staining Too Dark

1. Concentrated antibody not diluted out properly (being used at too high of a concentration).
2. Incubation of primary antibody or detection too long.

Figure 1:



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