Mouse-on-Mouse HRP-Polymer

Mouse Antibodies on Mouse Tissues
Polymer Detection Component
902-MM620-092019

BIOCARE MEDICAL

Catalog Number: **MM620 G, H, L, MM**

Description: 6.0, 25, 100mL, 1L

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

Summary & Explanation:
The Mouse-on-Mouse HRP-Polymer is specially designed for using mouse primary antibodies on mouse tissues with minimum cross-reactivity to endogenous mouse IgG. Utilization of this goat anti-mouse horseradish peroxidase (HRP) polymer is advantageous over traditional biotinylation methods. Biotinylated anti-mouse secondary antibodies can bind to endogenous mouse IgG in the tissue in addition to the primary antibody, leading to troublesome background staining. Biocare Medical's biotin-free polymer technology provides the elimination of endogenous biotin, reduction of IHC steps and increased sensitivity.

Biocare Medical has developed Rodent Block M for mouse tissues. It is a specially formulated blocking reagent that reduces nonspecific background staining and simultaneously blocks for endogenous mouse IgG. Rodent Block M is applied to tissue sections prior to the primary antibody for 30 minutes.

Biocare Medical has also developed Rodent Decloaker, an antigen retrieval solution, which helps reduce and/or eliminate endogenous mouse and rat IgG and nonspecific background staining. Temperature dependent protocols can be performed using Biocare Medical's Decloaking Chamber.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:
6mL
Mouse-on-Mouse HRP-Polymer (MM620G) 6mL
25mL
Mouse-on-Mouse HRP-Polymer (MM620H) 25mL
100mL
Mouse-on-Mouse HRP-Polymer (MM620L) 100mL
1L
Mouse-on-Mouse HRP-Polymer (MM620MM) 1L

Materials and Reagents Needed But Not Provided:
Microscope slides, positively charged
Desert Chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
Decloaking Chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*
Pretreatment reagents*
Enzyme digestion*
Peroxidase block*
Protein block*
Primary antibody*
Negative control reagents*
Chromogens*
Hematoxylin*
Bluing reagent*
Mounting medium*

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

Species Reactivity:
Mouse IgG heavy and light chains with minimum cross-reactivity on mouse tissues.

Storage and Stability:
Store 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:
1. Deparaffinize slides in Biocare's Slide Brite or xylene.
2. Hydrate slides in a series of graded alcohols to water.
3. **Optional:** Post-fix tissues in 10% formalin for 30 minutes. Wash in DI water. (See Technical Note #2).
4. Immerse slides in Biocare's Peroxidazed 1 blocking reagent for 3-5 minutes. Wash in DI water.
5. Place slides in 1X Rodent Decloaker and heat in Biocare's Decloaking Chamber.
Heating times can be used as follows:
- 80°C for 30-120 minutes or overnight for 12-18 hours
- 95°C for 30-60 minutes
- 110°C for 15 minutes
- 125°C for 30 seconds or 5 minutes at high temperature
Remove slides and wash in DI water.
6. **Digestion Technique (Optional):**
- Place slides in 1X Rodent Decloaker at 80°C for 15-30 minutes. Wash in DI water. A post digestion can be performed using pepsin at room temperature (RT) for 1-5 minutes. Wash in TBS wash buffer.
- If Rodent Decloaker is not necessary, apply pepsin at RT for 10-15 minutes. Wash in DI water.
- If using Rodent Decloaker at 95°C, 110°C or 125°C, a post-digestion can be performed using pepsin at RT for 30-60 seconds. Wash in TBS wash buffer.
7. **Blocking Step (Optional):**
Apply Rodent Block M for 30 minutes. Wash in TBS buffer. (See Technical Note #5)
8. Apply primary antibody for 30 minutes to 2 hours. Wash in TBS wash buffer. (See Technical Note #1)
9. Apply Mouse-on-Mouse HRP-Polymer for 10-20 minutes. Wash in TBS wash buffer. (See Technical Note #3)
10. **Chromogen:** Apply DAB for 5 minutes. Rinse in DI water.
11. Counterstain with hematoxylin. Wash in DI water. Apply Tacha's Bluing Solution for 1 minute. Wash in DI water.
12. Dehydrate, clear and coverslip.

Staining Protocol Recommendations (VALENT® Automated Slide Staining Platform):
Mouse-on-Mouse HRP-Polymer is compatible for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:
1. **Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.
2. **Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).
3. **Protein Block:** Incubate for 30 minutes at RT with Rodent Block M.
4. **Primary Antibody:** Incubate for 15 minutes.
5. **Polymer:** Incubate for 10-15 minutes with Mouse-on-Mouse HRP-Polymer.
6. **Chromogen:** Incubate for 5 minutes with Val DAB.
7. **Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

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Technical Notes:
1. Some primary mouse antibodies may not bind optimally with the secondary polymer; thus a longer incubation time with the primary antibody may be required.
2. Post-fixing tissue sections on slides for 15-30 minutes in 10% formalin reduces endogenous mouse IgG and helps prevent tissues from falling off the slides.
3. If endogenous mouse IgG is observed in tissue, apply the Mouse-on-Mouse HRP-Polymer for only 5-10 minutes. Double the incubation time of primary antibody and/or increase the antibody concentration to compensate for antibody staining.
4. Tissues such as colon or spleen may display background staining in the negative control. These kinds of tissues may require Biocare's MM Biotinylation Kit.
5. Biocare's Background Punisher can be used as a blocker for nonspecific background staining.

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 this reagent is Proclin 950 and the concentration is less than 0.25%. Overexposure to Proclin 950 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 950 in this product does not meet the OSHA criteria for a hazardous substance. 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.
8. 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. Staining steps performed incorrectly or in the wrong order.
3. Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, the wrong order or the wrong pretreatment.
4. Insufficient amount of antigen.
5. Primary antibody incubation period too short.
6. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining
1. Tissue is either over-fixed or under-fixed.
2. Primary antibody incubation too short.
3. Low expression of antigen.

Troubleshooting Guide Cont'd:
4. Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment.
5. Over-development of substrate.
6. Excessive rinsing during wash steps.
7. Omission of critical reagent.
8. Incorrect procedure in reagent preparation.
9. 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.

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