



Mouse-on-Mouse AP-Polymer

Mouse Antibodies on Mouse Tissues

Polymer Detection Component

Control Number: 902-MM624-012312

ISO
9001&13485
CERTIFIED

Catalog Number: MM624 G, H

Description: 6, 25 ml

Intended Use:

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

Summary & Explanation:

The Mouse-on-Mouse AP-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 alkaline phosphatase (AP) 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 AP-Polymer (MM624G) 6ml

25ml

Mouse-on-Mouse AP-Polymer (MM624H) 25ml

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*(TBS)

Pretreatment reagents*

Enzyme digestion*

Protein block*

Primary antibody*

Negative control reagents*

Chromogens*

Hematoxylin*

Bluing reagent*

Mounting medium*

* 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.

Species Reactivity:

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

Storage and Stability:

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

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. **Optional:** Immerse slides in Biocare's Peroxidized 1 blocking reagent for 3-5 minutes. Wash in DI water. (see Technical Note #5)
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
 - 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 used, apply pepsin at RT for 10-15 minutes. Wash in DI water.
 - If using Rodent Decloaker at 95°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 #6)
8. Apply primary antibody for 30 minutes to 2 hours. Wash in TBS wash buffer. (See Technical Note #1)
9. Apply Mouse-on-Mouse AP-Polymer for 10-20 minutes. Wash in TBS wash buffer. (See Technical Note #3)
10. **Chromogen:** Incubate for 5-7 minutes when using Biocare's Warp Red. 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.

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 AP-Polymer for only 10-15 minutes. Double the incubation time of primary antibody and/or increase the antibody concentration to compensate for antibody staining.
4. Use TBS wash buffer only. PBS wash buffers will inhibit alkaline phosphatase staining.
5. Blocking of endogenous peroxidase is not required; however immersing slides in Peroxidized 1 bleaches tissues, red blood cells and produces better contrast for alkaline phosphatase procedures.
6. Biocare's Background Punisher can be used as a blocker for nonspecific background staining.

Protocol Notes:

N/A





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Precautions:

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.

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. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request and is located at <http://biocare.net/support/msds/>.

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.

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.
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. Endogenous alkaline phosphatase.
3. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
4. Tissue may need a longer or a more specific protein block.
5. Substrate is overly-developed.
6. Tissue was inadequately rinsed.
7. Deparaffinization incomplete.
8. 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.

Performance Characteristics:

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

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

Limitations & Warranty:

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

Covered by one or more of the following US Pat. Nos. 6,686,461; 6,800,728; 7,102,024; 7,173,125; 7,462,689.

