Mouse-on-Mouse AP-Polymer

Micro-polymer detection
902-624-051724

BIOCARE MEDICAL

Available Product Formats	
Catalog Number	Volume
MM624G	6.0 mL
MM624H	25 mL
OPRR624T60	60 Tests

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

Background Information:
The Mouse-on-Mouse AP-Polymer is designed using a one-step method for detecting mouse primary antibodies to form an antibody-enzyme complex. This complex is then visualized using an appropriate substrate/chromogen. In the one-step method a secondary antibody directly linked to the polymer is applied. Mouse-on-Mouse AP-Polymer is provided ready-to-use.

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

Materials and Methods:

Reagents Provided:

Kit Catalog No.	Component Description	Quantity x Volume
MM624G	Mouse-on-Mouse AP-Polymer	1 x 6.0 mL
MM624H	Mouse-on-Mouse AP-Polymer	1 x 25 mL
OPRR624T60	Mouse-on-Mouse AP-Polymer	1 x 10.5 mL

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

Supplied As:
Buffered saline solution, pH 7.6-7.8, 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.

Reconstitution, Dilution and Mixing:
The Mouse-on-Mouse AP-Polymer kit reagent(s) are optimized and ready to use with Biocare IHC antibodies and ancillary reagents. No reconstitution, mixing, dilution, or titration is required.

Species Reactivity:
Mouse IgG heavy and light chains.

Storage and Stability:
Store at 2ºC to 8ºC. The product is 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 kit reagent(s) are ready-to-use and should not be diluted. The stability of user diluted reagent has not been established by Biocare.

Staining Protocol Recommendations (for intelliPATH FLX® and manual use):
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 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)

Staining Protocol Recommendations (for intelliPATH FLX® and manual use) Cont'd:
4. **Optional:** Immerse slides in Biocare's Peroxidazed 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
- 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 used, 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 #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.

Staining Protocol Recommendations (ONCORE™ Pro X Automated Slide Staining System):
The following programming and protocol recommendations are to assist the user when staining using Biocare's ONCORE Pro X Automated Staining Platform for research applications. The user is responsible for further optimizations of the protocol.

Mouse-on-Mouse AP-Polymer is provided in vials ready for use on the ONCORE Pro X Automated Slide Stainer. Uncap the vial and place in the ONCORE Pro X reagent tray. The ONCORE Pro X Automated Slide Stainer will apply reagent as required in the selected protocol. Refer to the ONCORE Pro X Automated Slide Staining System User Manual for detailed instructions on instrument operation and additional protocol options.

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 Peroxidazed 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.

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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. 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.
2. 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.[1,2]
3. 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.[3]
4. Microbial contamination of reagents may result in an increase in nonspecific staining.
5. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
6. Do not use reagent after the expiration date printed on the vial.
7. The reagent is optimized for use with Biocare antibodies and ancillary reagents. Refer to the primary antibody and other ancillary reagent instructions for use for recommended protocols and conditions for use.
8. Follow local and/or state authority requirements for method of disposal.
9. The SDS is available upon request and is located at http://biocare.net.

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 is 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. Overdevelopment of substrate.
6. Excessive rinsing during wash steps.
7. Omission of critical reagent.

Troubleshooting Guide Cont'd:
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. Tissue may need a longer or a more specific protein block.
3. Substrate is overly developed.
4. Tissue was inadequately rinsed.
5. Deparaffinization incomplete.
6. Tissue damaged or necrotic.

Troubleshooting Guide Cont'd:
Tissues Falling Off
1. Slides were not positively charged.
2. A slide adhesive was used in the water bath.
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.

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

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