

Mouse-on-Rat AP-Polymer

Mouse Antibodies on Rat Tissues
Detection Component
902-MRT623-032118

BIOCARE
M E D I C A L

Catalog Number: MRT623 H

Description: 25 ml

Intended Use:

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

Summary & Explanation:

Mouse primary antibodies on rat tissues are advantageous because mouse secondary detection systems can be used with minimum cross-reactivity to endogenous rat IgG.

Biocare Medical has developed a Mouse-on-Rat alkaline phosphatase (AP) polymer detection system with minimal cross-reactivity to rat tissues. It can be used with formalin-fixed paraffin-embedded tissues, floating sections, frozen sections and cell culture preparations.

Biocare Medical has novel products to help eliminate persistent rat IgG. XR Factor is a very potent blocker for eliminating endogenous rat IgG. By adding 1-2 drops (1 drop equals 33 µl) of the XR Factor to 2.5 ml of the Mouse-on-Rat Polymer, endogenous rat IgG will be dramatically reduced and/or completely eliminated. Rodent Block R is a blocking reagent for nonspecific background staining that also reduces endogenous rat IgG. Rodent Block R is applied to tissue sections prior to the primary antibody for 15-30 minutes. Biocare Medical has also developed Rodent Decloaker, an antigen retrieval solution, which helps reduce or eliminate endogenous IgG and non-specific background staining. Temperature dependent protocols can be performed using Biocare's Decloaking Chamber.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

Mouse-on-Rat AP-Polymer (MRT623H) 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*

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 information.

Species Reactivity:

Mouse IgG heavy and light chains with minimum cross-reactivity on rat 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 conditions other than those specified in the package insert, they must be verified by the user.

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 #3)

4. **Optional:** Immerse slides in Biocare's Peroxidized 1 blocking reagent for 3-5 minutes. Wash in DI water. (See Technical Note #6)

Staining Protocol Recommendations Cont'd:



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Rev. 062117

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5. **Optional:** Place slides in 1X Rodent Decloaker and heat to 80°C, 95°C, 110°C or 125°C using 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

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 R for 15-30 minutes. Wash in TBS buffer.

(See Technical Note #5)

8. Apply primary antibody for 30 minutes - 2 hours at RT or overnight at 2-8°C. Wash in TBS wash buffer. (See Technical Note #1)

9. Apply Mouse-on-Rat AP-Polymer for 20-30 minutes. Wash in TBS wash buffer. (See Technical Note #2)

10. **Chromogen:** Incubate for 5-7 minutes with 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. If endogenous rat IgG is observed in the negative control, add 1-2 drops of XR Factor (1 drop equals 33µl) to 2.5 ml of Mouse-on-Rat AP-Polymer and mix well.

3. Post-fixing tissue sections on slides for 15-30 minutes in 10% formalin reduces endogenous rat IgG and helps prevent tissues from falling off the slides.

4. Use TBS wash buffer only. PBS wash buffers will inhibit alkaline phosphatase staining.

5. Biocare's Background Punisher can be used as a blocker for nonspecific background staining.

6. Blocking for endogenous peroxidase is not required; however immersing slides in Peroxidized 1 bleaches tissues and red blood cells which produces better contrast for alkaline phosphatase staining procedures.

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 300 and the concentration is less than 0.25%. Overexposure to Proclin 300 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 300 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.

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Precautions Cont'd:

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. 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. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
3. Endogenous alkaline phosphatase (not blocked with levamisole).
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.

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.



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