Mouse-on-Rat AP-Polymer

Mouse Antibodies on Rat Tissues

Detection Component

Control Number: 902-MRT623-081917

Catalog Number: MRT623 G, H

Description: 6, 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:

6ml

Mouse-on-Rat AP-Polymer (MRT623G) 6ml

25ml

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 replaced 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*

* 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 rat tissues.

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 Peroxidazed 1 blocking reagent for 3-5 minutes. Wash in DI water. (See Technical Note #6)
5. **Optional:** Place slides in 1X Rodent Decloaker and heat to 80ºC, 95ºC or 125ºC 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
   - 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 95ºC or 125ºC, 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 2-8ºC. 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 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. 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 Peroxidazed 1 bleaches tissues and red blood cells which produces better contrast for alkaline phosphatase staining procedures.

Protocol Notes:

N/A

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:

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Mouse-on-Rat AP-Polymer

Mouse Antibodies on Rat Tissues

Detection Component

Control Number: 902-MRT623-081917

Storage and Stability:

Store at 2ºC to 8ºC. 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.

Precautions:

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.

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.

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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. Incorrect blocking reagent used; blocker should be from the same species in whihc 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.

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.

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