Rat-on-Mouse HRP-Polymer

Rat Primary Antibodies on Mouse Tissues

Polymer Detection Component

Control Number: 902-RT517-081617

Catalog Number: RT517 G, H, L

Description: 6, 25, 100 ml

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

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

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

The Rat-on-Mouse HRP-Polymer is comprised of two reagents: A Rat Probe that binds to the rat primary antibody and a Rat-on-Mouse HRP-Polymer that conjugates to the probe. This two-step system has been proven to be 10-20 times more sensitive than conventional conjugated mouse adsorbed anti-rat HRP secondary detection systems.

Biocare Medical has novel products to help eliminate persistent mouse IgG. XM Factor is a very potent blocker for eliminating endogenous mouse IgG. By adding 1-2 drops (1 drop equals 33µl) of the XM Factor to 2.5ml of the Rat-on-Mouse Polymer, endogenous mouse IgG will be dramatically reduced and/or completely eliminated. Rodent Block M is a blocking reagent for nonspecific background staining that also reduces endogenous mouse IgG. Rodent Block M is applied prior to the primary antibody for 15-30 minutes.

Biocare Medical has also developed Rodent Decloaker, an antigen retrieval solution, which helps reduce and/or eliminate endogenous mouse and rat IgG and 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
Rat Probe (RTP629G) 6ml
Rat-on-Mouse HRP-Polymer (RTH630G) 6ml

25ml
Rat Probe (RTP629H) 25ml
Rat-on-Mouse HRP-Polymer (RTH630H) 25ml

100ml
Rat Probe (RTP629L) 100ml
Rat-on-Mouse HRP-Polymer (RTH630L) 100ml

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*

* 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 the specific application and detection system used.

Species Reactivity:
Rat IgG with minimal cross-reactivity on mouse tissues.

Storage and Stability:
Store at 2ºC to 8ºC. 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 tissue in 10% formalin for 30 minutes. Wash in DI water. (See Technical Note #1)
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. (See Technical Note #4)
   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
   - 120-125˚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 and 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 #2)
8. Apply primary antibody for 30-60 minutes at RT or overnight at 2-8ºC. Wash in TBS wash buffer.
9. Apply the Rat Probe for 10-15 minutes. Wash in TBS wash buffer.
10. Apply the Rat-on-Mouse HRP-Polymer for 10-15 minutes. Wash in TBS wash buffer. (See Technical Note #3)
11. **Chromogen:** Apply DAB for 5 minutes. Rinse in DI water.
12. Counterstain with hematoxylin. Wash in DI water. Apply Tacha's Bluing Solution for 1 minute. Wash in DI water.
13. Dehydrate, clear and coverslip.

Technical Notes:
1. Post-fixing tissue sections on slides for 15-30 minutes in 10% formalin reduces endogenous mouse IgG and helps prevent tissues from falling off slides.
2. Biocare's Background Punisher can be used as a blocker for nonspecific background staining.
3. If endogenous mouse IgG is observed in tissue sections, add 1-2 drops (1 drop equals 33 µl) of XM Factor to 2.5 ml of Rat-on-Mouse HRP-Polymer.
4. This product is designed to work with 1X Rodent Decloaker. Other antigen retrieval solutions and/or protocols may cause non-specific background or insufficient staining.

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

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Polymer Detection Component

Control Number: 902-RT517-081617

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.
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. 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.

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.

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