Rabbit-on-Rodent HRP-Polymer

(Rabbit Antibodies on Mouse and Rat Tissues)
Polymer Detection
902-RMR622-092019

BIOCARE MEDICAL

Catalog Number: **RMR 622 G, H, L**

Description: 6.0, 25, 100 mL

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

Summary & Explanation:
Rabbit-on-Rodent HRP-Polymer is a one-step micro-polymer detection developed for use with rabbit monoclonal/polyclonal antibodies on mouse and rat tissue. Rabbit primary antibodies on mouse and rat tissues are advantageous because rabbit secondary detection systems can be used with minimum cross-reactivity to endogenous mouse and rat IgG.

Biocare Medical has developed a Rabbit HRP-Polymer detection system with minimal cross-reactivity to endogenous mouse and rat IgG. The advantages of this polymer technology are increased sensitivity, reduction of IHC steps and the elimination of endogenous biotin. It can be used with formalin-fixed paraffin-embedded tissues, floating sections, frozen sections and cell culture. Biocare's new rabbit polymer is ideal for single staining and can also be used in double and triple staining techniques.

In some tissues such as spleen, endogenous mouse or rat IgG may be difficult to eliminate. Biocare Medical has developed an antigen retrieval solution (Rodent Decloaker) that helps to reduce or eliminate endogenous IgG and non-specific background staining, and at the same time, performs antigen retrieval. Temperature dependent protocols can be performed using Biocare's Decloaking Chamber.

In addition, Biocare Medical has introduced new products to help eliminate persistent rat and mouse IgG. The XM and XR Factor are two potent blockers for eliminating endogenous mouse IgG and rat IgG, respectively. By adding 1-2 drops (1 drop equals 33µL) of the XM or XR Factor to 2.5 mL of rabbit polymer detection, endogenous mouse and/or rat IgG will be reduced dramatically or completely eliminated. Biocare has also developed Rodent Block M and Rodent Block R that block for non-specific background staining, and at the same time, reduce endogenous mouse and rat IgG, respectively. Rodent Block M or R is applied prior to the primary antibody for 15 to 30 minutes.

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

Supplied As:
6mL
Rabbit-on-Rodent HRP-Polymer (RMR622G) 6mL
25mL
Rabbit-on-Rodent HRP-Polymer (RMR622H) 25mL
100mL
Rabbit-on-Rodent HRP-Polymer (RMR622L) 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*(TBS/PBS)
Pretreatment reagents*
Enzyme digestion*
Peroxidase block*
Protein block*

Materials and Reagents Needed But Not Provided Cont'd:
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.

Species Reactivity:
Rabbit IgG heavy and light chains with minimum cross-reactivity on mouse and rat tissues.

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date.

Staining Protocol Recommendations:
1. Deparaffinize slides in Biocare's Slide Brite or xylene.
2. Hydrate slides in a series of graded alcohol to water.
3. **Optional:** Post-fix tissues in 10% formalin for 30 minutes (see Technical Note #1).
Wash in DI water.
4. Immerse slides in Biocare's Peroxidazed 1 blocking reagent for 3-5 minutes. Wash in DI water.
5. **Optional:** Place slides in 1X Rodent Decloaker and heat to 80°C, 95°C, 110°C or 125ºC using Biocare's Decloaking Chamber. Heating times can be used as following (see Technical Note #2):
- 80°C for 30 to 120 minutes or overnight for 12 to 18 hours
- 95°C for 30 to 60 minutes
- 110°C for 15 minutes
- 125°C for 30 seconds or 5 minutes at high temperature
6. Remove slides and wash in DI water
7. **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 for 2 to 5 minutes. Wash in TBS wash buffer.
- If Rodent Decloaker is not necessary, apply pepsin at room temperature for 10-15 minutes and 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 room temperature for 30-60 seconds. Wash in TBS wash buffer.
8. **Optional:** If troublesome background staining occurs, apply Rodent Block M (on mouse tissue) or Rodent Block R, (on rat tissue) for 15-30 minutes to reduce nonspecific background staining and/or endogenous mouse or rat IgG. Wash in TBS buffer
9. Apply primary antibody for 30 to 60 minutes at room temperature or overnight at 2-8ºC. Wash in TBS wash buffer (see Technical Note #3).
10. Apply Rabbit-on-Rodent HRP-Polymer for 20-30 minutes. Wash in TBS wash buffer (see Technical Note #4).
11. Apply DAB for 5 minutes.
12. Rinse in DI water
13. Apply CAT Hematoxylin for 30 seconds to 1 minute or Tacha's Automated Hematoxylin for 5 minutes. Wash in DI water.
14. Apply Tacha's Bluing Solution for 1-2 minutes. Wash in DI water
15. Dehydrate, clear and coverslip.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

Rev: 062117

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Staining Protocol Recommendations (VALENT® Automated Slide Staining Platform):

Rabbit-on-Rodent HRP-Polymer is compatible for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

1. **Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.
2. **Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Lo pH, 5X (use at 1X).
3. **Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.
4. **Protein Block:** Incubate for 10 minutes at RT with Val Background Block.
5. **Primary Antibody:** Incubate for 30 minutes.
6. **Polymer:** Incubate for 30 minutes with Rabbit-on-Rodent HRP-Polymer.
7. **Chromogen:** Incubate for 5 minutes with Val DAB.
8. **Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

Technical Notes:

1. Post-fixing tissue sections on slides for 15-30 minutes in 10% formalin (apply after step #2) reduces endogenous mouse and rat IgG and helps prevent tissues from falling off the slides.
2. 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.
3. Biocare's Background Punisher can be used as a diluent to reduce non-specific background staining.
4. If endogenous mouse or rat IgG is observed in tissue sections, add 1-2 drops (1 drop equals 33µL) of XM or XR Factor to 2.5 mL of Rabbit-on-Rodent HRP-Polymer.

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. 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.
8. 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. 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.

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