Rat HRP-Polymer, 1-Step (Mouse adsorbed)

Detection of Rat Primary Antibodies on Mouse Tissues
902-4016-050119

BIOCARE MEDICAL

Catalog Number: **BRR4016 H**

Description: 25 mL

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

Summary & Explanation:
Biocare's PromARK™ series introduces a 1-step horseradish peroxidase (HRP) polymer detection system for IHC of rat primary antibodies on mouse tissues, with minimal cross-reactivity.

Rat HRP-Polymer, 1-Step (Mouse adsorbed) offers the convenience and sensitivity of a one-step polymer detection system that is biotin and avidin free. It is specifically formulated to improve specificity and reduce background staining on mouse tissues.

The elimination of mouse IgG can be a persistent problem in achieving optimal staining of mouse tissues. In addition to the 1-step Rat HRP-Polymer (Mouse adsorbed) detection, Biocare offers reagents that may further reduce unwanted background staining. Adding XM Factor directly to Rat HRP-Polymer may further reduce background staining due to endogenous mouse IgG. Rodent Block M may also be used to reduce nonspecific background staining (see individual data sheets).

Biocare has also developed Rodent Decloaker, an antigen retrieval solution, which is specifically formulated to reduce and/or eliminate non-specific background staining due to endogenous mouse and rat IgG. Temperature dependent antigen retrieval protocols can be performed using Biocare's Decloaking Chamber (pressure cooker).

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

Supplied As:
Rat HRP-Polymer, 1-Step (Mouse adsorbed) (BRR4016H) 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*
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. Certain reagents listed above are based on 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. 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 xylene or xylene substitute (Biocare's Slide Brite).
2. Hydrate slides in a series of graded alcohols to water.
3. Immerse slides in Biocare's Peroxidazed 1 blocking reagent for 3-5 minutes. Wash in DI water.
4. Place slides in 1X Rodent Decloaker and perform heat-induced antigen retrieval in Biocare's Decloaking Chamber. (See Technical Note #1)
   Suggested heating protocols for antigen retrieval include:
   - 80˚C for 30-120 minutes (or overnight for 12-18 hours)
   - 95˚C for 30-60 minutes
   - 110˚C for 5-15 minutes
   - 125˚C for 30 seconds

   Alternatively, antigen retrieval may be performed for 5 minutes in boiling solution. Remove slides and wash in DI water after heating.
5. Digestion Technique (Optional): Digestion may be optionally performed before or after antigen retrieval.
   - If digestion is performed before retrieval, pepsin at RT for 10-15 minutes (or 37ºC for 5 minutes), followed by a TBS wash is recommended.
   - If digestion is performed after retrieval, pepsin at RT for 1-5 minutes (when retrieval was 80°C) or 30-60 seconds (when retrieval was 95°C or 125°C), followed by a TBS wash is recommended.
6. Blocking Step (Optional): Apply Rodent Block M for 15-30 minutes. Wash in TBS buffer. (See Technical Note #2)
7. Apply primary antibody for 30-60 minutes at RT or overnight at 2-8ºC. Wash in TBS wash buffer.
8. Apply Rat HRP-Polymer for 30 minutes. Wash in TBS wash buffer. (See Technical Note #3)
9. Chromogen: Apply DAB for 5 minutes. Rinse in DI water.
10. Counterstain with hematoxylin. Wash in DI water. Apply Tacha's Bluing Solution for 1 minute. Wash in DI water.
11. Dehydrate, clear and coverslip.

Technical Notes:

1. Rat HRP-Polymer, 1-Step has been developed for optimal performance when staining tissues retrieved with Rodent Decloaker. Other antigen retrieval solutions and/or protocols may cause non-specific background or insufficient staining.
2. Biocare's Background Punisher may also be used as a blocker for nonspecific background staining.
3. If background staining due to 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 HRP-Polymer.
4. Make sure tissue sections do not dry out after antigen retrieval.

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.

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USA

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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. 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. Tissue drying-out after antigen retrieval
3. Primary antibody incubation too short
4. Low expression of antigen
5. Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment
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. 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

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

Over Staining
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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