Mouse-on-Canine AP-Polymer

Mouse Antibodies on Canine and Feline Tissues

Polymer Detection

Control Number: 902-4003-101717

Catalog Number: BRR 4003 G, H, L

Description: 6.0, 25, 100 ml

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

Summary & Explanation:
Biocare's PromARK™ series introduces Mouse-on-Canine AP-Polymer Detection. This detection is specially designed for using mouse antibodies on canine and feline tissues and utilizes biotin-free alkaline phosphatase (AP) polymer technology. The advantages of this polymer technology are increased sensitivity, reduction of IHC steps (no Avidin/Biotin block or Link/Probe), and minimal cross-reactivity to endogenous canine and feline IgG.

The Mouse-on-Canine detection can be used with paraffin-embedded tissues and can be performed manually or on automated staining platforms. The addition of proprietary blockers to the detection reagent permits the use of any of Biocare Medical's retrieval solutions (Reveal/Borg/Diva) or enzymes with the elimination of endogenous IgG or non-specific background staining. Temperature dependent protocols can be conveniently performed using Biocare's Decloaking Chamber.

The Mouse-on-Canine AP-Polymer may be combined with the Rabbit-on-Canine HRP-Polymer to create a double stain polymer detection that will label the mouse antibody with AP and the rabbit antibody with HRP. Refer to Technical Notes #5 and #6 for additional information.

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

Supplied As:

6ml
Mouse-on-Canine AP-Polymer (BRR4003G) 6ml
25ml
Mouse-on-Canine AP-Polymer (BRR4003H) 25ml
100ml
Mouse-on-Canine AP-Polymer (BRR4003L) 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 specific application and detection system used.

Species Reactivity:
Mouse IgG heavy and light chains with minimum cross-reactivity on canine and feline tissues.

Storage and Stability:
Store at 2ºC to 8ºC. Do not use after expiration date printed on vials. If reagents are stored under 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:** Place slides in 1X retrieval solution and heat to 80˚C, 95˚C or 125ºC using Biocare's Decloaking Chamber. Heating times can be used as following (see Technical Note #1):
   - 80˚C for 30-120 minutes, or for improved morphology, 60˚C-70˚C overnight for 12-18 hours
   - 95˚C for 30-60 minutes
   - 125˚C for 30 seconds or 5 minutes
4. Remove slides and wash in DI water.
5. **Optional:**Digestion Technique
- Place slides in 1X retrieval solution at 80˚C for 15-30 minutes. Wash in DI water.
- A post digestion can be performed using an enzyme at room temperature (RT) for 2-5 minutes. Wash in TBS wash buffer.
- If a retrieval solution is not necessary, apply enzyme at RT for 10-15 minutes and wash in DI water.
- If using a retrieval solution at 95˚C or 125˚C, a post digestion can be performed using an enzyme at RT for 30-60 seconds. Wash in TBS wash buffer.
6. Apply primary antibody for 30-60 minutes at RT or overnight at 2ºC-8ºC. Wash in TBS wash buffer.
7. **Optional:** Blocking Step: Incubate with a protein block for 10 minutes if background staining is noted. (see Technical Note #2)
8. Apply Mouse-on-Canine AP-Polymer for 30-60 minutes. Wash in TBS wash buffer.
9. Chromogen: Incubate for 5-7 minutes when using Biocare's Warp Red.
10. Counterstain with hematoxylin. Rinse with DI water. Apply Tacha's Bluing Solution for 1 minute. Rinse with DI water.
11. Dehydrate, clear and coverslip.

Technical Notes:
1. This product is designed to work with no pre-treatment, Biocare's 1X retrieval solutions or enzymes. Please check the antibody datasheet. Other antigen retrieval solutions and/or protocols may cause non-specific background or insufficient staining.
2. Biocare's Background Punisher can be used as an optional protein block and is recommended to be applied prior to detection if background staining is noted.
3. This detection can also be used on an automated staining system.
4. Use TBS wash buffer only. PBS wash buffers will inhibit alkaline phosphatase staining.
5. To prepare a double stain polymer detection, combine equal volumes of the Mouse-on-Canine AP-Polymer and the Rabbit-on-Canine HRP-Polymer. Apply to tissue for 30-60 minutes at step #8 in the protocol. Apply the HRP chromogen prior to applying the AP chromogen to complete the double stain. Store at 2°C to 8°C. The expiration date of the combined polymer detection will be the earliest expiration date of the individual components.
6. If performing a double stain polymer detection, after step #2 in the protocol, immerse slides in Biocare's Peroxidazed 1 blocking reagent for 3-5 minutes. Wash in DI water.

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.

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Mouse Antibodies on Canine and Feline Tissues

Polymer Detection

Control Number: 902-4003-101717

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. 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 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.

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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