Rabbit-on-Farma AP-Polymer

Rabbit Antibodies on Bovine, Equine, Porcine, Ovine & Avian Tissues

Affinity Purified Polymer Detection

Control Number: 902-BRR4011-112713

Catalog Number: BRR4011 G, H

Description: 6.0, 25ml

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

Summary & Explanation:
Biocare's PromARK™ series includes Rabbit-on-Farma AP-Polymer detection. The Rabbit-on-Farma AP-Polymer is formulated to detect rabbit polyclonal or monoclonal antibodies and is affinity purified for minimal cross-reactivity to cow, horse, pig, sheep, chicken and swan IgG proteins. The advantages of this polymer technology are increased sensitivity, reduction of IHC steps (no avidin/biotin block or link/enzyme conjugates), minimal cross-reactivity to endogenous IgG that allows multiple species flexibility, and in most cases, tissues do not require a protein block. The Rabbit-on-Farma detection can be used on paraffin-embedded tissues and can be performed manually or on automated staining platforms. This detection may be used with any of Biocare Medical's retrieval solutions (Reveal/Borg/Diva) or digestive enzymes. Temperature dependent protocols can be conveniently performed using Biocare's Decloaking Chamber.

The Rabbit-on-Farma AP-Polymer may be combined with the Mouse-on-Farma HRP-Polymer to prepare a double stain polymer detection that will label the rabbit antibody with AP and the mouse antibody with HRP. Refer to Technical Note #4 for additional information.

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

Supplied As:

6ml
Rabbit-on-Farma AP-Polymer (BRR4011G) 6ml

25ml
Rabbit-on-Farma AP-Polymer (BRR4011H) 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*

* 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:
Rabbit IgG heavy and light chains with minimum cross-reactivity on cow, horse, pig, sheep, chicken and swan 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.

Staining Protocol Recommendations:

1. Deparaffinize slides in Biocare's Slide Brite or xylene.
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. **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 at high temperature
5. Remove slides and wash in DI water.
6. **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 to 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 TBS wash buffer.
- 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.
7. Apply primary antibody for 30-60 minutes at RT or overnight at 2-8ºC. Wash in TBS wash buffer.
8. **Optional:** Blocking Step: Incubate with a protein block for 10 minutes (see Technical Note #2).
9. Apply Rabbit-on-Farma AP-Polymer for 30-60 minutes. Wash in TBS wash buffer.
10. Apply Warp Red for 7 minutes. Rinse in DI water.
11. Counterstain with hematoxylin. Rinse with deionized water.
12. Apply Tacha's Bluing Solution for 1-2 minutes. Wash in DI water.
13. 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 antibody data sheet. 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 the detection if background staining is noted.
3. The polymer can also be used on an automated staining system.
4. To prepare a double stain polymer detection, combine equal volumes of the Rabbit-on-Farma AP-Polymer and the Mouse-on-Farma HRP-Polymer. Apply to tissue for 30 -60 minutes at step #9 in the protocol. After the HRP chromogen application, apply an 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.
5. Use TBS wash buffer only. PBS wash buffers will inhibit alkaline phosphatase staining.

Staining Protocol Notes:
N/A

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.

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

tel: 800-799-9499 fax: 925-603-8080 www.biocare.net

BIOCARE MEDICAL, LLC.
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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 MSDS is available upon request and is located at http://biocare.net/support/msds/.
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. 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.

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