

MACH 2 Double Stain 1

Mouse-AP + Rabbit-HRP

Polymer Detection Kit

Control Number: 902-523-100617

Catalog Number: BRR 523 G, H, L

Description: 6.0, 25, 100ml

Intended Use:

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

Summary & Explanation:

The conjugated goat anti-mouse polymer alkaline phosphatase (AP) and the conjugated goat anti-rabbit polymer horseradish peroxidase (HRP) secondary antibodies react with both heavy and light chains on mouse and rabbit IgG respectively. In-house studies have shown this innovative AP- and HRP-polymerization technology provides a significant increase in staining sensitivity when compared to other conventional AP- or HRP-conjugated secondary antibodies. Avidin-biotin blocking procedures are not necessary when using the MACH 2 conjugated secondary antibodies. It is specially designed for a rapid double stain procedure. An antibody cocktail with a rabbit monoclonal/polyclonal and a mouse monoclonal antibody must be used. The overall staining procedure can be done in 4 steps and in less than two hours. It can be used manually or used with automated stainers.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

6ml

MACH 2 Double Stain 1 (BRR523G) 6ml

25ml

MACH 2 Double Stain 1 (BRR523H) 25ml

100ml

MACH 2 Double Stain 1 (BRR523L) 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)
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 and Rabbit IgG heavy and light chains

Storage and Stability:

Store at 2°C to 8°C. 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:

Deparaffinization: Deparaffinize slides in Slide Brite or xylene. Hydrate slides in a series of graded alcohols to water.

Peroxide Block: Block for 5 minutes with Biocare's Peroxidized 1.

Pretreatment Solution/Protocol: Please refer to the respective primary antibody data sheet for recommended pretreatment solution and protocol.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody Cocktail: Please refer to the respective primary antibody data sheet for incubation time.

Polymer: Incubate for 20-30 minutes at RT with MACH 2 Double Stain 1.

Chromogen (1): Incubate for 5 minutes at RT with Biocare's Betazoid DAB.

Chromogen (2): Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Notes:

1. Use Biocare's 20X TBS wash buffer. Do not use PBS-based wash buffers!

Protocol Notes:

The optimum antibody dilution and protocols for a specific application can vary due to many factors. These include, but are not limited to: fixation, incubation times, tissue section thickness and detection kit used. The data sheet's recommendations and protocols are based on exclusive use of Biocare products. 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. This reagent has not been validated with heat (37-42°C). Ultimately, it is the responsibility of the investigator to determine optimal conditions.

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 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.

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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. Secondary antibody at too low of a concentration.
6. Primary antibody incubation period too short.
7. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining

1. Tissue is either over-fixed or under-fixed.
2. Primary antibody incubation time 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. Endogenous alkaline phosphatase (not blocked with levamisole).
3. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
4. Tissue may need a longer or a more specific protein block.
5. Substrate is overly-developed.
6. Tissue was inadequately rinsed.
7. Deparaffinization incomplete.
8. 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.