MACH 4 Universal HRP-Polymer Detection System

Polymer Detection

Universal Blocker, DAB Chromogen

Control Number: 902-BRR4012-110117

Catalog Number: BRR 4012 G, H, L

Description: 6.0, 25, 100 ml

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

Summary & Explanation:
The MACH 4 Universal HRP-Polymer Detection System has been developed for use with mouse and rabbit primary antibodies. This biotin-free technology uses a specific probe to detect mouse primary antibodies, which is then followed by a horseradish peroxidase (HRP) polymer that binds to the probe on to rabbit antibodies. This innovative HRP polymerization technology provides increased staining sensitivity when compared to Envision + and other polymer detection kits. It can be used manually and on automated stainers.

3, 3' Diaminobenzidine (DAB) is a widely used chromogen for immunohistochemical staining and immunoblotting. In the presence of peroxidase enzyme, DAB produces a brown precipitate that is insoluble in alcohol and xylene. This product comes in a two-component system consisting of a liquid stable DAB chromogen and DAB substrate buffer. The color of the chromogen buffer mixture may vary from colorless to pale brown. Staining properties are not affected by the color of the chromogen buffer mixture. Betazoid DAB is a superior formulation that is very stable. In some cases, antibody titers may increase as much as two-fold. Betazoid DAB can be used both manually and on automated stainers.

Background Punisher is a universal blocking reagent used for reducing nonspecific background staining often found with immunohistochemistry. Background Punisher is specifically formulated for superior pH stability and is sodium azide and thimerosal free. It can be used with automated or manual staining protocols.

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

Supplied As:

6 ml Kit
MACH 4 Mouse Probe (BRR534DG) 6ml
MACH 4 HRP-Polymer (BRR534BG) 6ml
Betazoid DAB Chromogen (BRR900AE) 0.25ml
Betazoid DAB Substrate Buffer (BRR900BG) 6ml
Background Punisher (BRR974G) 6ml
Mixing vial (VL103) 1ea

25 ml Kit
MACH 4 Mouse Probe (BRR534DH) 25ml
MACH 4 HRP-Polymer (BRR534BH) 25ml
Betazoid DAB Chromogen (BRR900AC) 1ml
Betazoid DAB Substrate Buffer (BRR900BH) 25ml
Background Punisher (BRR974H) 25ml
Mixing vial (VL103) 1ea

100 ml Kit
MACH 4 Mouse Probe (BRR534DL) 100ml
MACH 4 HRP-Polymer (BRR534BL) 100ml
Betazoid DAB Chromogen (BRR900AF) 4ml
Betazoid DAB Substrate Buffer (BRR900BL) 100ml
Background Punisher (BRR974L) 100ml
Mixing vial (VL103) 1ea

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*)

Materials and Reagents Needed But Not Provided cont'd:
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 and Rabbit IgG heavy and light chains

Storage and Stability:
Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

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 Peroxidazed 1.
Pretreatment Solution/Protocol: Please refer to the respective primary antibody datasheet for recommended pretreatment solution and protocol.
Protein Block (Optional): Incubate for 5-10 minutes at room temperature (RT) with Biocare's Background Punisher.
Primary Antibody: Please refer to the respective primary antibody datasheet for incubation time.
Probe: (mouse antibodies only) Incubate for 5-15 minutes at RT with MACH 4 Mouse Probe.
Polymer: Incubate for 10-30 minutes at RT with MACH 4 HRP-Polymer. (Recommendation: 10-15 minutes for mouse primary antibodies; 30 minutes for rabbit antibodies.)
Chromogen:
1. Mix 1 drop (32ul) of Betazoid DAB Chromogen per 1.0ml of Betazoid DAB Substrate Buffer. The DAB working solution is stable for 5 days if stored at 2-8°C.
2. Apply DAB mixture to tissue sections. Incubate for 2-5 minutes at RT.
Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Notes:

1. Primary antibody titers can be dramatically increased when using Biocare's Revival Series Diluents and Heat Retrieval Solutions.
2. Do not use goat serum as a protein block.
3. MACH 4 is at least 3 times stronger than MACH 3 when comparing mouse monoclonal antibodies. Biocare's monoclonal predilute protocols can be reduced to 15-10-10, except for ER and PR.
4. The rabbit portion of MACH 4 is not as strong as MACH 3. We recommend a staining protocol of 30-45 minutes for most of Biocare's rabbit predilutes; and a 30 minute incubation for the polymer. No probe is required for rabbit antibodies.
5. MACH 3 and MACH 4 may be used for in situ hybridization procedures using a mouse specific antibody against specific probes such as biotin. Preliminary data has shown extremely high sensitivity and detection of targets with low copy expression.
6. Use TBS for washing steps.

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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. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

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

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

Precautions:

This product is not classified as hazardous. The preservative used in this reagent is Proclin 950 and the concentration is less than 0.25%. Overexposure to Proclin 950 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 950 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.

Warnings:

1. DAB is known to be a suspected carcinogen.
2. Do not expose DAB components to strong light or direct sunlight.
3. Wear appropriate personal protective equipment and clothing.
4. DAB may cause sensitization of skin. Avoid contact with skin and eyes.
5. Observe all federal, state and local environmental regulations.

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.

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. 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. 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).

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