

## MACH 4 Universal HRP-Polymer

Biotin-Free Detection  
Polymer Detection Kit  
901-M4U534-072817

**BIOCARE**  
M E D I C A L

**Catalog Number:** M4U534 G, H, L, MM, G80

**Description:** 6.0, 25, 100 ml, 1L, 80 ml

### Intended Use:

For In Vitro Diagnostic Use

MACH 4 Universal HRP-Polymer is a horseradish peroxidase (HRP)-antibody conjugate system intended for use in the detection of mouse IgG and IgM, and rabbit IgG primary antibodies on formalin-fixed, paraffin-embedded (FFPE) tissues in an immunohistochemistry (IHC) procedure. The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

### Summary & Explanation:

The Universal HRP-Polymer detects both mouse and rabbit antibodies. This biotinfree technology uses a specific probe to detect mouse primary antibodies and is then followed by a horseradish peroxidase polymer (HRP) that binds to the probe. This innovative HRP-polymerization technology provides increased staining sensitivity for mouse monoclonal antibodies when compared to Envision + and other polymer detection kits. It can be used manually and on automated stainers.

### Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

### Supplied As:

#### 6ml

MACH 4 Mouse Probe (UP534G) 6ml  
MACH 4 HRP-Polymer (MRH534G) 6ml

#### 25ml

MACH 4 Mouse Probe (UP534H) 25ml  
MACH 4 HRP-Polymer (MRH534H) 25ml

#### 100ml

MACH 4 Mouse Probe (UP534L) 100ml  
MACH 4 HRP-Polymer (MRH534L) 100ml

#### 1L

MACH 4 Mouse Probe (UP534MM) 1L  
MACH 4 HRP-Polymer (MRH534MM) 1L

### G80 (for the intelliPATH Automated Slide Stainer)

MACH 4 Mouse Probe (UP534G20) 20ml x 4  
MACH 4 HRP-Polymer (MRH534G20) 20ml x 4

### 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\*

### Materials and Reagents Needed But Not Provided Cont'd:

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 vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

### 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 room temperature (RT) with Biocare's Background Punisher.

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

**Probe (mouse antibodies only):** Incubate for 5-15 minutes at RT with MACH 4 Mouse Probe.

**Polymer:** Incubate for 10-20 minutes for mouse antibodies or 30 minutes for rabbit antibodies at RT with MACH 4 HRP-Polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB.

**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. Use TBS for washing steps.

### Limitations:

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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

## MACH 4 Universal HRP-Polymer

Biotin-Free Detection  
Polymer Detection Kit  
901-M4U534-072817

**BIOCARE**  
M E D I C A L

### 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:

1. 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.
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.

### Troubleshooting:

Follow the reagent specific protocol recommendations according to the 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. 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, link or label too long.