

# MACH 4™ Universal AP Polymer Kit

Biotin-Free Detection

Polymer Detection Kit Control

Number: 902-536C-091117

**Catalog Number:** BRR536C G, H, L, G20

**Description:** 6.0, 25, 100 ml, 20ml

**Intended Use:**

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

**Summary & Explanation:**

The Universal AP Polymer Kit detects both mouse and rabbit primary antibodies. This biotin-free technology uses a specific probe to detect mouse primary antibodies and is then followed by an alkaline phosphatase polymer (AP) that binds to the probe. This innovative AP-polymerization technology provides increased staining sensitivity up to 5 to 20 times when compared to Envision + and other AP-polymer detection kits. It can be used manually and on automated stainers.

Covered by one or more of the following US Pat. Nos. 6,686,461; 6,800,728; 7,102,024; 7,173,125; 7,462,689.

**Known Applications:**

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:**

**6ml Kit**

MACH 4 Universal AP Probe (BRR536BG) 6ml

MACH 4 MR AP Polymer (BRR536AG) 6ml

**25ml Kit**

MACH 4 Universal AP Probe (BRR536BH) 25ml

MACH 4 MR AP Polymer (BRR536AH) 25ml

**100ml Kit**

MACH 4 Universal AP Probe (BRR536BL) 100ml

MACH 4 MR AP Polymer (BRR536AL) 100ml

**G20 Kit (for the IntelliPATH Automated Slide Stainer)**

MACH 4 Universal AP Probe (BRR536BG20) 20ml

MACH 4 MR AP Polymer (BRR536AG20) 20ml

**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 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 any 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 (Optional):** Block for 5 minutes with Biocare's Peroxidized I.

**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 to 15 minutes at RT with MACH 4 Universal AP Probe.

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

**Chromogen:** 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 TBS wash buffer only. PBS wash buffers will inhibit alkaline phosphatase staining.
2. Primary antibody titers can be dramatically increased when using Biocare's Revival Series Diluents and Heat Retrieval Solutions.
3. Do not use goat serum as a protein block. Do not use Biocare's Background Eraser or Background Terminator.

**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 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.

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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, link or label too long.