MACH 1 Universal HRP-Polymer Detection

Biotin-Free Detection Polymer Detection Kit
901-M1U539-080717

Catalog Number: **M1U539 G, L10**

Description: 6.0, 110 ml

Intended Use:

For In Vitro Diagnostic Use
MACH 1 Universal HRP-Polymer Detection is intended for laboratory use in the qualitative detection of mouse IgG, mouse IgM, and rabbit primary antibodies in immunohistochemistry (IHC) procedures on formalin-fixed paraffin-embedded (FFPE) tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using 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:

This new biotin-free technology uses a specific probe to detect mouse antibodies followed by a HRP-polymer that binds to both the probe and rabbit antibodies. Avidin, biotin and normal serum blocking steps are eliminated. This innovative HRP-polymerization technology provides increased staining sensitivity for mouse and rabbit antibodies when compared to other one-step polymers.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity:

Mouse IgG, Mouse IgM and Rabbit IgG heavy and light chains

Supplied As:

6 ml Kit

MACH 1 Mouse Probe (UP537G)
MACH 1 Universal HRP-Polymer (MRH538G)
Background Sniper (BS966G)
Betazoid DAB Chromogen (BDB900B)
Betazoid DAB Substrate Buffer (DS900G)
Mixing Vial (MV539)

110 ml Kit

MACH 1 Mouse Probe (UP537L10)
MACH 1 Universal HRP-Polymer (MRH538L10)
Background Sniper (BS966)
Betazoid DAB Chromogen (BDB900G)
Betazoid DAB Substrate Buffer (DS900L10)
Mixing Vial (MV539)
MACH 1 Mouse Probe Dropper Bottle (DB537)
MACH 1 Universal HRP-Polymer Dropper Bottle (DB538)
Background Sniper Dropper Bottle (DB966)
Betazoid DAB Dropper Bottle (DB900)

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/PBS)
Pretreatment reagents*
Enzyme digestion*
Peroxidase block*
Primary antibody*
Negative control reagents*
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.

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 enzyme pretreatment solution and protocol. Note: For most antibodies Diva Decloaker is recommended. Borg Decloaker may be used for antigens with low expression levels.

Protein Block (Optional):

Incubate for 10-15 minutes at room temperature (RT) with Biocare's Background Sniper.

Primary Antibody:

Please refer to the respective primary antibody datasheet for incubation time.

Probe (No probe for rabbit antibodies):

Incubate for 15 minutes at RT with MACH 1 Mouse probe (mouse monoclonals only).

HRP-Polymer:

Incubate for 30 minutes at RT for both mouse and rabbit antibodies.

Chromogen: When using Biocare's Betazoid DAB:

1. Mix 1 drop (32ul) of DAB Chromogen per 1.0ml of 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 5 minutes.
3. Rinse tissue with PBS or TBS Wash Buffer or deionized water as appropriate.

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 or horse serum as a protein block.
3. To help facilitate workflow, the probe can be applied on rabbit antibodies. Apply probe for 5-15 minutes. Rinse in buffer and apply HRP-polymer for 30 minutes.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

IVD CE

Rev: 062117

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

MACH 1 Universal HRP-Polymer Detection

Biotin-Free Detection Polymer Detection Kit
901-M1U539-080717

Limitations:
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. 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.

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.

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) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment.
4. Insufficient amount of antigen.
5. Primary antibody incubation period too short.
6. Secondary antibody at too low of a concentration.
7. 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. Endogenous alkaline phosphatase or peroxidase (not effectively blocked).
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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080