MACH 2 Universal HRP-Polymer Detection

Mouse and Rabbit Polymer-HRP Secondary Antibody

Polymer Detection Kit

Control Number: 901-M2U522-090617

Catalog Number: M2U522 G, H, L

Description: 6.0, 25, 100 ml

Intended Use:
For In Vitro Diagnostic Use

Summary & Explanation:
The conjugated goat anti-mouse and anti-rabbit polymer-horseradish peroxidase secondary antibody reacts with heavy and light chains on mouse and rabbit IgG. It reacts with all mouse and rabbit IgG subclasses and with mouse and rabbit IgM. The new and innovative HRP-polymerization technology provides a significant increase in staining sensitivity when compared to other conventional HRP-conjugated secondary antibodies. Avidin-biotin blocking procedures are not necessary when using the MACH 2 conjugated secondary antibodies. The overall staining procedure uses one less reagent (streptavidin peroxidase) and rinsing step, eliminates avidin-biotin blocking procedures, and may reduce overall staining time. It can be used manually and on automated stainers.

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

Supplied As:
Buffer with protein carrier and preservative

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*
Avidin-Biotin Blocking Kit*(Labeled Streptavidin Kits Only)
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.

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 alcohol 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:
Incubate for 10-15 minutes at RT with BIOCARE's Background Sniper.

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

Polymer:
Incubate for 30 minutes at RT with MACH 2 Universal HRP-Polymer.

Chromogen:
Incubate for 5 minutes at RT when using BIOCARE's DAB.

Technical Notes:

1. Primary Antibody titers can be dramatically increased when using BIOCARE's Revival Series Diluents and Heat Retrieval Solutions. (Talk to your BIOCARE Sales Representative)
2. We recommend BIOCARE's TBS Wash Buffer for wash and rinsing steps.
3. Use TBS for washing steps.

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.

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 NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on Tissue Controls.

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Precautions:

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.

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.

Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

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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 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. Variable fixation time.
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.

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