

## MACH 2 Double Stain 2

Mouse-HRP + Rabbit-AP

Polymer Detection Kit

Control Number: 901-MRCT525-081817

**Catalog Number:** MRCT525 G, H, L

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

**Intended Use:**

For In Vitro Diagnostic Use

**Summary & Explanation:**

The conjugated goat anti-mouse polymer horseradish peroxidase (HRP) and the conjugated goat anti-rabbit polymer alkaline phosphatase (AP) secondary antibodies react with both heavy and light chains on mouse and rabbit IgG respectively. This innovative HRP- and AP-polymerization technology provides a significant increase in staining sensitivity when compared to other conventional HRP- or AP-conjugated secondary antibodies. Avidin-biotin blocking procedures are not necessary when using the MACH 2 conjugated secondary antibodies. It is specially designed for a rapid double stain procedure. An antibody cocktail with a mouse monoclonal and a rabbit polyclonal/monoclonal must be used. The overall staining procedure can be done with 4 major steps and in less than two hours. It can be used manually or used with automated stainers.

**Supplied As:**

**6ml**

MACH 2 Double Stain 2 (MRCT525G) 6ml

**25ml**

MACH 2 Double Stain 2 (MRCT525H) 25ml

**100ml**

MACH 2 Double Stain 2 (MRCT525L) 100ml

**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)
- 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 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 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 Cocktail:** Please refer to the respective primary antibody datasheets for incubation time.

**Polymer:** Incubate for 20-30 minutes at RT with MACH 2 Double Stain 2.

**Chromogen (1):** Incubate for 5 minutes at RT when using Biocare's Betazoid DAB.

**Chromogen (2):** Incubate for 5-7 minutes at RT when using 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 Biocare's 20X TBS wash buffer. Do not use PBS-based wash buffers!

**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. This reagent has not been validated with heat (37-42°C). 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

**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.

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.

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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 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 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.