

**Mouse-&-Rabbit-on-Rodent Double Stain Polymer**  
**Mouse Antibodies (HRP) & Rabbit Antibodies (AP) on Rat and Mouse Tissues**  
**Polymer Detection Component**  
 Control Number: 902-RDS513-082417

**Catalog Number:** RDS513 H  
**Description:** 25 ml

**Intended Use:**

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

**Summary & Explanation:**

The Mouse-&-Rabbit-on-Rodent Double Stain Polymer is specifically designed for a rapid double stain procedure of primary mouse and rabbit antibodies on mouse or rat tissue. This simultaneous double stain procedure can be done in approximately two hours, compared to approximately four hours for a sequential double stain. This single solution is a cocktail of conjugated goat anti-mouse horseradish peroxidase (HRP) polymer and conjugated goat anti-rabbit alkaline phosphatase (AP) polymer which reacts simultaneously with a cocktail of mouse and rabbit primary antibodies. The polymer technology is biotin-free and thus provides the elimination of endogenous biotin and reduction of IHC steps (no avidin-biotin blocking). This innovative cocktail of HRP- and AP-polymer technology provides a significant increase in staining sensitivity when compared to other conventional HRP or AP conjugated secondary antibodies. The double stain polymer detection can be used manually or on an automated stainer.

Biocare Medical has developed Rodent Block M (for mouse tissues) or Rodent Block R (for rat tissues) blocking reagents that reduce nonspecific background staining and endogenous mouse or rat IgG. Rodent Block is applied to the tissue prior to the primary antibody for 15-30 minutes.

Biocare Medical has also developed Rodent Decloaker, an antigen retrieval solution, which helps reduce and eliminate endogenous mouse and rat IgG and non-specific background staining while simultaneously performing antigen retrieval. Temperature dependent protocols can be performed using Biocare's Decloaking Chamber.

**Known Applications:**

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:**

**25ml**  
 Mouse-&-Rabbit-on-Rodent Double Stain Polymer (RDS513H) 25ml

**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 with minimum cross-reactivity on mouse and rat tissues.

**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:**

1. Deparaffinize slides in Biocare's Slide Brite or xylene.
2. Hydrate slides in a series of graded alcohols to water.
3. **Optional:** Post-fix tissues in 10% formalin for 30 minutes. Wash in DI water. (See Technical Note #2).
4. Immerse slides in Biocare's Peroxidized 1 blocking reagent for 3-5 minutes. Wash in DI water.
5. Place slides in 1X Rodent Decloaker and heat in Biocare's Decloaking Chamber. Heating times can be used as follows:
  - 80°C for 30-120 minutes or overnight for 12-18 hours
  - 95°C for 30-60 minutes
  - 125°C for 30 seconds or 5 minutes at high temperature
 Remove slides and wash in DI water.
6. **Digestion Technique (Optional):**
  - Place slides in 1X Rodent Decloaker at 80°C for 15-30 minutes. Wash in DI water. A post digestion can be performed using pepsin at room temperature (RT) for 1-5 minutes. Wash in TBS buffer.
  - If Rodent Decloaker is not used, apply pepsin at RT for 10-15 minutes. Wash in DI water.
7. **Blocking Step:**  
 Apply Rodent Block M (mouse tissue) or Rodent Block R (rat tissue) for 30 minutes. Wash in TBS buffer. (See Technical Note #3)
8. Apply antibody cocktail for 30-60 minutes or overnight at 2-8°C. Wash in TBS buffer.
9. Apply Mouse-&-Rabbit-on-Rodent Double Stain Polymer for 30 minutes. Wash in TBS buffer.
10. **Chromogen:**  
 Apply DAB for 5 minutes. Rinse in DI water. Incubate for 5-7 minutes with Biocare's Warp Red. Rinse in DI water.
11. Counterstain with hematoxylin. Wash in DI water. Apply Tacha's Bluing Solution for 1 minute. Wash in DI water.
12. Dehydrate, clear and coverslip.

**Technical Notes:**

1. Use TBS buffer only. PBS-based buffers will inhibit alkaline phosphatase staining.
2. Post-fixing tissue sections on slides for 15-30 minutes in 10% formalin reduces endogenous mouse or rat IgG and helps prevent tissues from falling off the slides.
3. Biocare's Background Punisher can be used as a blocker for nonspecific background staining.

**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.

# Mouse-&-Rabbit-on-Rodent Double Stain Polymer

## Mouse Antibodies (HRP) & Rabbit Antibodies (AP) on Rat and Mouse Tissues

### Polymer Detection Component

Control Number: 902-RDS513-082417

**Precautions:**

1. 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.
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 (1).
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.

**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. Primary antibody incubation period too short.
6. 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 (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 or detection too long.

**References:**

1. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.