

# intelliPATH™ Multiplex Secondary Reagent 2

Mouse-HRP + Rabbit-AP

Polymer Detection

Control Number: 901-IPSC5004-091411

**Catalog Number:**

IPSC 5004 G20  
IPSC 5004 G80

**Description:**

20 ml, Ready-to-use  
80 ml, Ready-to-use

**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. The innovative HRP- and AP-polymerization technology provides a significant increase in staining sensitivity when compared to conventional HRP- or AP-conjugated secondary antibodies. Avidin-biotin blocking procedures are not necessary when using the multiplex conjugated secondary antibodies.

The intelliPATH™ Multiplex Secondary Reagent 2 is designed for use on the intelliPATH automated stainer, in conjunction with a cocktail consisting of a mouse monoclonal antibody and a rabbit polyclonal/monoclonal antibody. This method provides a rapid, 4-step staining procedure that can be completed in less than two hours.

**Known Applications:**

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:**

intelliPATH™ Multiplex Secondary Reagent 2 (IPSC5004G20) 1x20ml  
intelliPATH™ Multiplex Secondary Reagent 2 (IPSC5004G80) 4x20ml

**Materials and Reagents Needed But Not Provided:**

- Microscope barrier slides, positively charged
- Desert chamber\* (Drying oven)
- Positive and negative tissue controls
- Xylene (Could be replaced with a 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 media\*

\* 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.

**Pretreatment Solution/Protocol:** Please refer to the respective prediluted multiplex cocktail datasheet for recommended pretreatment solution and protocol.

**Peroxide Block:** Block for 5 minutes at room temperature (RT).

**Primary Antibody Cocktail:** Please refer to the respective prediluted multiplex cocktail datasheet for incubation time.

**Secondary:** Apply intelliPATH Multiplex Secondary Reagent 2. Incubate for 15-30 minutes at RT.

**Chromogen (1):** Apply intelliPATH DAB for 5 minutes.

**Chromogen (2):** Apply intelliPATH Fast Red (15-20 min) or intelliPATH Warp Red (5-7 min).

**Counterstain:** Rinse with deionized water. Incubate for 5 minutes with automated Hematoxylin. Rinse with TBS Buffer for 1 minute followed by a rinse with deionized water.

**Technical Notes:**

1. Use TBS wash buffer. PBS-based wash buffers will inhibit alkaline phosphatase staining.

**Protocol Notes:**

N/A

**Quality Statement:**

Biocare protocols have been standardized using Biocare antibodies, detection and ancillary reagents on the intelliPATH automated stainer. Recommended staining protocols are specified in the datasheet of the antibody of interest. Pre-optimized intelliPATH protocols with preset parameters can be displayed, printed and edited according to the procedure in the operator's manual. Refer to the operator's manual for additional instruction to navigate intelliPATH software and stainer.

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



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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 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 or poorly processed.

##### 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.

### 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.

