

## 4plus Streptavidin HRP Label

Conjugated Streptavidin Horseradish Peroxidase  
Detection Component  
901-HP604-031418

**BIOCARE**  
M E D I C A L

**Catalog Number:** HP604 H, L

**Description:** 25, 100 ml

### Intended Use:

For In Vitro Diagnostic Use

4plus Streptavidin HRP Label is a horseradish peroxidase (HRP)-antibody conjugate system intended for use in the detection of biotinylated secondary or primary antibodies on formalin-fixed, paraffin-embedded (FFPE) tissues in an immunohistochemistry (IHC) procedure. The clinical interpretation of any staining or its absence should be complemented by morphological studies and 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:

Streptavidin is a protein that has similar binding properties to egg white avidin. It is isolated from streptomyces avidinii. Streptavidin has a molecular weight of 60 kDa and has 4 subunits. Each subunit can bind one molecule of biotin. Biotin is a water-soluble vitamin. Streptavidin has an extremely high binding affinity ( $K_d=10^{-15}$ ) for biotin. Streptavidin conjugated to horseradish peroxidase has proven useful in the detection of biotinylated secondary or primary antibodies directed to their specific epitopes.

There are several advantages when using a streptavidin-conjugates versus an ABC complex. In contrast to avidin, streptavidin is not glycosylated and is therefore uncharged at neutral pH (6.5 versus 10). This lowers nonspecific background staining. Streptavidin also lacks carbohydrate side chains that may be another cause of non-specific background. Another key advantage of streptavidin is the significant increase in sensitivity (probably due to less steric hindrance), thus facilitating an increase in overall binding capacity.

Streptavidin-HRP is much more stable than the ABC complex. The ABC complex must be freshly made 30 minutes prior to use and is stable only for a few days. In contrast, the Streptavidin-HRP reagent is very stable, color-coded, and can be stored for up to 1-2 years. Biocare's Streptavidin-HRP comes in a ready-to-use format, thus saving time and potential mistakes.

### Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

### Supplied As:

Peroxidase-conjugated streptavidin in PBS 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)

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\*

\* Refer to the Biocare Medical website located at <http://biocare.net> for information regarding catalog numbers and ordering information.

### Species Reactivity:

N/A

### 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 data sheet for recommended pretreatment solution and protocol.

### Avidin/Biotin Block:

Incubate for 10-20 minutes at RT with Biocare's Avidin.

Incubate for 10-20 minutes at RT with Biocare's Biotin.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

**Primary Antibody:** Please refer to the respective primary antibody data sheet for incubation time.

**Link:** Incubate for 10 minutes at RT with 4plus Biotinylated Link.

**Label:** Incubate for 10-20 minutes at RT with 4plus Streptavidin HRP Label.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB.

**Counterstain:** Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

### Technical Note:

Use TBS for washing steps.

### Limitations:

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. 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.

### 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](http://www.clsi.org)). 2011

### Precautions:

1. 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.



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### Precautions Cont'd:

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.
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.

### 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.