

4plus Streptavidin AP Label

Conjugated Streptavidin Alkaline Phosphatase
901-AP605-071717

BIOCARE
M E D I C A L

Catalog Number: AP 605 H, L

Description: 25, 100 ml

Intended Use:

For In Vitro Diagnostic Use

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 alkaline phosphatase has proven useful in the detection of antigens coupled with biotinylated secondary antibodies.

There are several advantages when using a streptavidin-conjugate 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 nonspecific 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-ALP 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-ALP reagent is very stable, color-coded, and can be stored for up to 1-2 years. Biocare's Streptavidin-ALP comes in a ready-to-use format, thus saving time and potential mistakes.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

Alkaline phosphatase-conjugated streptavidin is diluted in TBS with protein carrier and preservative (sodium azide free).

Materials and Reagents Needed But Not Provided:

Microscope 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* (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 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:

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 alcohol 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:

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.

Link:

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

Label:

Incubate for 10 minutes at RT with 4plus Streptavidin-AP Label.

Chromogen:

Incubate for 10-20 minutes at RT when using Biocare's Vulcan Fast Red.

Counterstain:

Rinse with deionized water. Incubate for 30-60 seconds with Tacha's Automated Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing solution for 1 minute.

Technical Notes:

1. A hydrogen peroxide blocking solution may be used to bleach red blood cell. Apply before pretreatment protocols and/or before the blocking serum step. It will have no effect on the alkaline phosphatase system.

2. Use TBS for washing steps. Do not use PBS wash buffers with alkaline phosphatase systems.

Protocol Notes:

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

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Performance Characteristics Cont'd:

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

Precautions:

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. 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. 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.

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.