

# Avidin-Biotin Kit

Blocking Reagent  
901-AB972-071117

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

**Catalog Number:** AB972 H, L, M  
**Description:** 25, 100, 500 ml, Ready-to-use

## Intended Use:

For In Vitro Diagnostic Use

The Avidin-Biotin Kit is comprised of A and B solutions and is intended to reduce background staining caused by endogenous biotin on formalin-fixed, paraffin-embedded (FFPE) tissues in immunohistochemistry (IHC) procedures. 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:

The Avidin and Biotin solutions are color-coded with a pH indicator. In most cases, endogenous biotin in tissue sections is masked by formalin fixation. However, if avidin-biotin IHC detection systems are used with frozen sections or tissues pretreated with a heat-induced, epitope retrieval method (HIER), an avidin-biotin blocking technique may be needed. It can also be used on an automated staining system.

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

## Supplied As:

### 25ml Kit

Avidin (AB972H-A) 25ml  
Biotin (AB972H-B) 25ml

### 100ml Kit

Avidin (AB972H-A) 25ml x 4  
Biotin (AB972H-B) 25ml x 4

### 500ml Kit

Avidin (AB972M-A) 500ml  
Biotin (AB972M-B) 500ml

## 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\*  
Peroxidase block\*  
Primary antibody\*  
Negative control reagents\*  
Detection kits\*  
Detection components\*  
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.

## 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

## Protocol Recommendations:

1. After incubation with normal serum, incubate section or cells with Avidin Solution for 10-20 minutes.
2. Rinse with wash buffer.
3. Incubate tissue or cells for 10-20 minutes with the Biotin Solution.
4. Rinse in 3 changes of wash buffer.

These steps can be performed either prior to application of the primary antibody, or can be performed after the application of primary antibody. In some cases, the application of these solutions prior to or after the primary antibody can block specific staining. For strong endogenous biotin staining, longer incubation times with the avidin-biotin solutions may be required.

Some tissues, such as kidney, liver, lung, spleen, breast and brain may contain endogenous biotin. To detect endogenous biotin staining, cut two additional slides from the same block and test with the following procedure:

Slide 1:

A) Pretreat tissue or cells in the normal way and omit the primary and secondary antibody.

B) Apply a streptavidin-conjugate and a substrate to the tissue or cells.

Slide 2:

A) Pretreat the tissue or cells identically to slide 1.

B) Apply only the substrate to the tissue.

Lightly counterstain with hematoxylin (5 seconds), dehydrate, clear and coverslip. Observe slides under a microscope. If there is no background staining in slide 2, then the background staining in slide 1 is most likely due to endogenous biotin.

## Protocol Notes:

The blocking kit consists of equal amounts of an Avidin solution (pale pink) and of a Biotin solution (pale blue). Pretreatment of tissues with the Avidin solution should always be followed by incubation with the Biotin solution. If the Avidin solution turns from a pale pink to orange or yellow, the pH of the solution has changed and it should not be used. If the Biotin solution turns from a pale blue to green or yellow, the pH of the solution has changed and should not be used. The correct color of these solutions will assure the end-user that proper pH and optimum performance is being maintained.

## 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

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## Limitations Cont'd:

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.
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 antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.