



Avidin-Biotin Kit

Blocking Reagent

Control Number: 902-972A-121812

Catalog Number: BRR 972 AH, AL, AM

Description: 25, 100, 500 ml, Ready-to-use

Intended Use:

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

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	500ml Kit
Avidin (BRR972BH)	Avidin (BRR972BM)
Biotin (BRR972CH)	Biotin (BRR972CM)

100ml Kit
Avidin (BRR972BH)x4
Biotin (BRR972CH)x4

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 fuchsia, or 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 a royal blue, or turns 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:

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.

Precautions:

1. This product is not classified as hazardous. The preservative used in this reagent is Proclin 950 and the concentration is less than 0.5%. 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 MSDS is available upon request and is located at <http://biocare.net/support/msds/>.
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.

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.