VECTOR LABORATORIES

PRODUCT INFORMATION

Product AVIDIN/BIOTIN BLOCKING KIT

Catalog No. SP-2001

Amount 18 ml of each solution

Storage Conditions 4 °C

Blocking kit reagents may be used to block nonspecific binding of Biotin/Avidin System reagents.

Principle:

Some tissues may bind avidin, biotinylated horseradish peroxidase or other Biotin/Avidin System components without prior addition of biotinylated antibody. This binding may be due to endogenous biotin or biotin-binding proteins, lectins, or nonspecific binding substances present in the section. If a high background is present using the ABC reagents (or other avidin conjugate) in the absence of biotinylated secondary antibody, pre-treatment of the tissue with avidin, followed by biotin (to block the remaining biotin binding sites on the avidin), may be required.

The blocking kit consists of an Avidin D solution and a biotin solution. Pre-treatment of the section with the Avidin D solution should always be followed by incubation with the biotin solution. The Avidin D and biotin solutions should be used directly as supplied.

Suggested Protocol for Tissue Sections:

After incubation with normal serum, incubate section with Avidin D solution for 15 minutes. Rinse briefly with buffer, then incubate for 15 minutes with the biotin solution. These steps should be performed prior to the addition of primary antibody or lectin.

In many cases an alternative procedure has proved satisfactory. This method incorporates avidin/biotin blocking into the normal steps employed in labeling. Four drops of the Avidin D solution can be added to each 1 ml of the diluted normal blocking serum (preferably dialyzed to remove any free biotin from the serum). This reagent is used in place of the usual serum block step. After a brief rinse, the primary antibody is added, containing 4 drops of the biotin solution per 1 ml of primary antibody. This step not only introduces the primary antibody into the section, but blocks the available biotin binding sites on the avidin. Combining the biotin block step with the primary antibody step is not recommended if the primary antibody is biotinylated. When using biotinylated primary antibodies, the biotin solution should be added prior to the addition of primary antibody as a separate step.

Suggested Protocol for Transfer Blots:

After the initial blocking step with 1x casein, the membrane is immersed for 10 minutes in a dilute avidin solution prepared by dispensing 2 drops of the Avidin D solution into 10 ml of TBS (using the protein blocking protocol). Wash briefly with buffer. Incubate the membrane for 10 minutes in a dilute biotin solution, made by dispensing 2 drops of the Biotin solution into 10 ml of TBS. Proceed with the transfer blot detection procedure.

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