Double Immunofluorescent Procedure Using Two Biotinylated Secondary Antibodies and Avidin D Fluorochrome Conjugates

(Fluorescein and Texas Red)

(Procedure for deparaffinized or fixed frozen sections)

1. Incubate sections for 20 minutes with diluted normal blocking serum which was prepared from the species in which the secondary antibody is made.
2. Blot excess serum from sections.
3. Incubate sections for 30-60 minutes with the first primary antibody (follow protocol suggested by supplier of primary antibody).
4. Wash slides for 5 minutes in buffer.
5. Incubate sections for 30 minutes with diluted biotinylated secondary antibody solution.
6. Wash slides for 5 minutes in buffer.
7. Incubate sections with Fluorescein Avidin D for 20-30 minutes.
8. Wash slides for 5 minutes with buffer.
9. Apply the Avidin / Biotin Blocking kit (follow instructions supplied with the kit).
10. Incubate sections for 20 minutes with diluted normal blocking serum.
11. Blot excess serum from sections.
12. Incubate sections for 30-60 minutes with second primary antibody (follow protocol suggested by supplier of primary antibody).
13. Wash slides for 5 minutes in buffer.
14. Incubate sections for 30 minutes with diluted biotinylated secondary antibody solution.
15. Wash slides for 5 minutes in buffer.
16. Incubate sections with Texas Red Avidin D for 20-30 minutes.
17. Wash slides for 5 minutes with buffer.
18. Mount with VECTASHIELD® mounting media.
19. Observe under fluorescence microscope.

**NOTE: This is a general protocol for applications in which two biotinylated secondary antibodies are used. Modifications may be necessary for different applications.