Protocol: Double Immunofluorescent Labeling Using Two Primary Antibodies From Different Species

Staining for First Antigen

1. Preparation of tissue. Fix sections with the appropriate fixative for the antigen under study (Please see Note 1).

2. Air dry sections.

3. Wash sections 2 x 2 minutes in buffer (PBS).

4. Avidin/biotin blocking step. Perform Avidin/Biotin blocking if required (Avidin/Biotin Blocking Kit, Cat. No. SP-2001). Incubate sections with Avidin Solution for 15 minutes. Rinse briefly with buffer, then incubate in the Biotin Solution for 15 minutes. Wash sections 2 x 2 minutes in buffer. This blocking step may be eliminated if suitable controls have determined this step to be unnecessary.

5. Protein blocking step. Incubate sections for 20 minutes with buffer containing 5% normal blocking serum (NS) which was prepared from the species in which the secondary antibody is made.

6. Blot excess serum from sections.

7. Primary antibody. Incubate sections with the first primary antibody diluted in appropriate antibody diluent (buffer containing 5% NS) using an appropriate concentration and length of incubation.

8. Wash for 5 minutes in buffer.

9. Secondary antibody. Incubate sections for 30 minutes with biotinylated secondary antibody (5-10 µg/ml diluted in buffer containing 5% NS).

10. Wash slides for 5 minutes in buffer.

11. Avidin conjugate. Incubate sections with Fluorescein Avidin DCS (15-20 µg/ml diluted in buffer) for 5-10 minutes.

12. Wash slides for 5 minutes in buffer.

Staining for Second Antigen

13. Avidin/biotin blocking step. Apply an Avidin/Biotin block according to Step 4. (This step must be done to prevent the interaction of the second set of labeling reagents with the first set of labeling regents).

14. Protein blocking step. Incubate sections for 20 minutes with 5% NS.

15. Blot excess serum from sections.

16. Primary antibody. Incubate sections with second primary antibody diluted in appropriate antibody diluent (buffer containing 5% NS) using an appropriate concentration and length of incubation.

17. Wash slides for 5 minutes in buffer.

18. Secondary antibody. Incubate sections for 30 minutes with biotinylated secondary antibody (5-10 µg/ml diluted in buffer containing 5% NS).

19. Wash slides for 5 minutes in buffer.

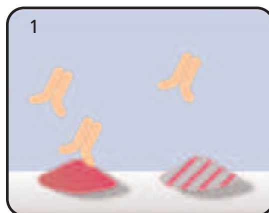
20. Avidin conjugate. Incubate sections with Texas Red® Avidin DCS (15-20 µg/ml diluted in buffer) for 5-10 minutes.

21. Wash slides for 5 minutes in buffer.

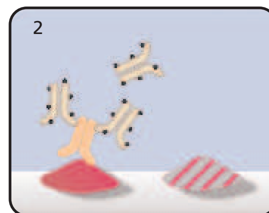
22. Mount with the appropriate VECTASHIELD® mounting media.

23. Observe under a fluorescence microscope.

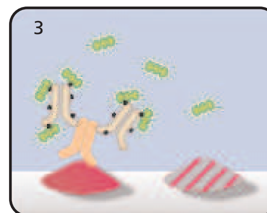
NOTES:
1. Aldehyde-fixed tissues (e.g. formalin) tend to be autofluorescent and may make interpretation of specific fluorescein signal difficult.



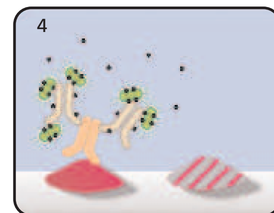
Add first primary antibody.



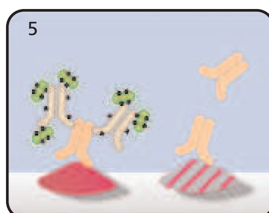
Add biotinylated secondary antibody.



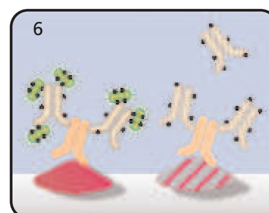
Add Fluorescein Avidin DCS.



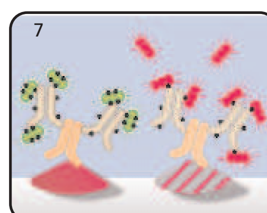
Add avidin/biotin block.



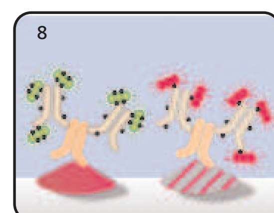
Add second primary antibody.



Add biotinylated secondary antibody.



Add Texas Red® Avidin DCS.



Mount and observe.