Protocol for Immunostaining Fixed and Paraffin Embedded Tissue Sections

Note: This protocol is designed for staining formalin fixed, paraffin embedded tissue sections.

1. Incubate slides in a dry oven at 60°C for 1 hour. Slides should be maintained in a vertical orientation to allow complete removal of the paraffin. Allow the slide to cool to room temperature.
2. Deparaffinize by immersing the slides in xylene (5 times for 4 min).
3. Hydrate slides by immersing the slides in 100%, 95%, 70% ethanol, 30% ethanol (2 times for 3 min each), and distilled or deionized water for 5 min.
4. Before incubation with the antibodies, antigen retrieval must be performed. Immerse the slides in citrate buffer (0.01M, pH 6.0).
5. Microwave (700W or high) for 5 min, add citrate buffer if necessary
6. Microwave on medium for 5 min, add citrate buffer if necessary.
7. Microwave on low for 5 min.
8. After cooling, transfer to PBS (pH 7.4; 2 times for 5 min each),
9. Incubate sections with 3% hydrogen peroxide for 20 minutes to eliminate endogenous peroxidase activity.
10. Rinse sections with PBS (5 times for 2 min each).
11. Incubate sections with 10% normal horse serum (Vector Laboratories) for 20 min in a humidified chamber at room temperature.
12. Treat with primary antibody (diluted 1/500 in PBS) and allowed to sit overnight at 4 °C.
13. Rinse sections with PBS (3 times for 5 min each).
14. Treat slide with a biotinylated secondary anti-mouse antibody (2 hrs to overnight) and then rinse in PBS (3 times for 5 min each).
15. Incubate sections with the chromogen DAB (from Vectastain ABC Elite kit, Vector Laboratories) for 2-10 min, depending on the intensity of staining desired.
16. Stop the reaction by washing in distilled water.
17. Counterstain slides in Mayer’s hematoxylin for 10 sec.
18. Dehydrate slides in 30%, 70%, 95%, and 100% ethanol.
19. Clear slides in xylene (4 times for 5 min each).
20. Mount cover slide with Permount.

View through a microscope.