Suggested Immunohistochemistry Protocol for REDI-PRO™ Tissue Sections

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Note: Do not let the tissues dry out once they are re-hydrated.
Use separate tubs for antibodies and negative control slides.

MATERIALS

REDI-PRO™ tissue slides
Coverslips
Slide racks
Staining dishes with lids
Plastic slide tray (Baxter Scientific Cat. No. M6304 or similar)
Orbital shaker
PAP pen
Transfer pipettes

Deionized water (DI H_2O)
PBS (Phosphate Buffered Saline)
Hydrogen peroxide (H_2O_2)
Primary antibody
Biotinylated secondary antibody, HRP conjugated
Bovine Serum Albumin (BSA – for blocking)
Streptavidin-HRP
DAB
Hematoxylin (optional)
Acetic Acid (optional)
Paramount Coverslip solution

BUFFERS

Working Citrate Buffer
9mL of 0.1M Citric Acid (10.5g citric acid monohydrate to 500mL DI H_2O)
41mL of 0.1M Sodium Citrate (14.7g sodium citrate dehydrate to 500mL DI H_2O)
450mL of DI H_2O

Deparaffinization and Rehydration (Cover staining dishes with a lid in each step)

1. Dip slides in three (3) changes of xylene or a xylene substitute for 3 minutes each.
2. Dip slides in two (2) change of 100% alcohol for 3 minutes each.
3. Dip slides in one (1) change of 95% alcohol for 3 minutes.
4. Dip slides in one (1) change of 70% alcohol for 3 minutes.
5. Rinse slides twice (2x) in DI H_2O for 5 minutes.

Antigen Retrieval (Microwave Method)

6. Soak slides in 3% H_2O_2 for 5 minutes.
7. Rinse slides twice (2x) in DI H_2O for 5 minutes.
8. Soak the slides in the working citrate buffer and cover with a lid.
9. Microwave until the liquid boils, about 1-5 minutes.
10. Remove from heat and let it stand at room temperature for 20 minutes.

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11. Wash three (3) times for 5 minutes in DI H_2O
12. Remove the liquid (do not touch the tissue!) and use a PAP pen to circle around the tissue.

Blocking

13. Apply enough 5% BSA with a transfer pipette to cover the tissues.
14. Incubate the slides overnight at 4°C in a humid chamber.

Primary Antibody

15. Dilute the primary antibody to the recommended concentration in 1% BSA/PBS diluent.
16. Remove the BSA, and incubate with primary antibody solution for 1 hour at room temperature.
17. Wash slides three (3) times 5 minutes each on the shaker.

Secondary Antibody and Detection

18. Dilute the biotinylated secondary antibody to 1:200 in 1% BSA diluent.
19. Incubate with secondary antibody solution for 30 minutes at room temperature.
20. Wash slides in PBS three (3) times 5 minutes each on the shaker.
21. Add enough streptavidin HRP to cover the tissues. Incubate for 30 minutes at room temperature
22. Wash three (3) times 5 minutes each in PBS on the shaker.
23. Add enough DAB to cover the tissues. Once the cells start turning brown (inexperienced technicians may wish to observe this under a microscope), wash twice (2x) in PBS for 5 minutes each on the shaker.

Optional Counterstain

24. Dip the slide rack with the slides into a staining dish of hematoxylin for 30 seconds.
25. Dip into an acetic bath (200mL DI H_2O with one to three drops of acetic acid). Rinse with DI H_2O.

Dehydration (Cover staining dishes with a lid in each step)

26. Dip slides in 70% and 95% alcohol for 3 minutes each.
27. Dip slides in 2 changes of 100% alcohol for 3 minutes.
28. Dip slides in 3 changes of xylene or xylene substitute for 3 minutes.

Cover Slips

29. Drizzle Paramount coverslip solution onto coverslips or slides.
30. Apply coverslip to slide.
31. Let the slides dry overnight.