Immunohistochemistry Protocol - General

Day 1

Deparaffinize

1. Dip slides in 100% Citrisolv 3 x 5mins

Hydration

2. Dip in 100% EtOH 2 x 2mins
3. Dip in 95% EtOH 2 x 2mins
4. Dip in 80% EtOH 2 x 2mins
5. Dip in ddH2O 2 x 2mins

Quench endogenous peroxides

6. Incubate slides in 0.3% H2O2 in methanol for 15mins (500uL of 30% H_2O_2 in 50mL MetOH, in white containers)

Antigen retrieval – Use citrate buffer pH 6.0 **or** Tris-EDTA buffer pH 9.0 based on antigen desired

7. Pre-heat antigen retrieval solution in microwave in white containers (~50mL), for 5mins at full power. Use large plastic container and fill halfway with water
8. Transfer slides to white containers with AR solution. Microwave for 10mins at power level 1
9. Rest slides at room temperature for 20mins, change water in bottom of container

Wash

10. Remove AR solution and wash slides with ddH_2O for 5 mins
11. Draw bubble with hydrophobic pen during this wash
12. Rinse slides with PBS for 5mins

Blocking

13. Block with 5% normal goat serum in PBS + 0.1% Triton-x-100 + 0.05% Tween-20, 30 minutes @ RT (300uL/slide)

Primary antibody

14. Dilute antibody in 5% normal goat serum in PBS + 0.1% Triton-x-100 + 0.05% Tween-20, overnight at 4°C (300uL/slide)

Day 2

Wash

15. Rinse with PBS; 3 x 5 minutes

Secondary antibody

16. Add secondary antibody diluted 1:225 in PBS + 0.1% triton-X + 0.05% tween-20 with 5% normal goat serum (~300uL/slide)
    a. Commonly: biotinylated anti-rabbit IgG (H+L) made in goat (Vector Labs, BA-1000) or biotinylated anti mouse IgG (H+L) made in goat (Vector Labs, BA-9200)
17. Incubate at room temperature for 1-2 hours

Wash

18. Rinse with PBS; 3 x 5 minutes

Amplification

19. Prepare amplification solution at least 30mins before use
20. Amplification with VECTASTAIN® Elite® ABC HRP Kit
    a. 9uL solution A per mL PBS, vortex; + 9uL solution B per mL PBS, vortex; (300uL/slide)
    b. Incubate at room temperature for 1-2hours

Wash

21. Rinse slides with PBS; 3 x 5 minutes

Substrate development – Use DAB **or** eDAB depending on antigen being detected

22. *For regular DAB:*
    a. Add 1mL of DAB to 150mL of 50mM Tris + 50uL 30% H_2O_2
    b. Dip slides into DAB solution, develop until colour appears ~ 3 minutes.
23. *For eDAB (SIGMAFAST™ DAB with Metal Enhancer):*
    a. Add 1 tablet DAB/Cobalt and 1 tablet Urea Hydrogen Peroxide to 5mL ddH_2O, vortex until dissolved; **prepare immediately before use.**
    b. Use ~300uL/slide, develop until colour appears ~ 3 minutes.
24. Rinse with PBS to stop reaction
25. Rinse with ddH_20 for 5mins

Counterstain

26. Counterstain with 1:2 Hematoxylin:ddH_2O, dip slides in quickly
27. Remove excess hematoxylin in ddH_2O; 2X

Dehydration

28. 5 dips, 1 minute each:
    a. 50% EtOH
    b. 80% EtOH x2
    c. 95% EtOH
    d. 100% EtOH x2
    e. 1:1 Citrisolv: 100% EtOH
    f. 100% Citrisolv x2

Coverslip

29. Coverslip with permount solution, allow slides to dry before viewing on microscope