Impress IHC

**Detection System:** Vector ImmPRESS kits and ImmPACT DAB

Incubate slide @ 600C O/N.

Use appropriate kit for primary antibody host.

Check DAB exposure time for your antibody, or determine DAB time.

1. Dewax x 2 in xylene, 5 mins
2. Dehydrate through ethanol: 100%, 100%, 90%, 70%, 5 mins. Wash slides in dH20, 5 mins
3. Peroxidase quenching in 1:30 H2O2 to 1XPBS, 30 mins (10ml 30% H2O2:290ml PBS). Turn on the steamer approximately 17 minutes into H2O2 treatment.
4. Place CB in steamer with a foil lid to warm (30ml 10X stock citrate buffer: 270ml H2O)
5. dH2O wash, 5 mins.
6. Place slides in warm 1X CB, 20 mins, then cool in ice bucket (15 mins - until slides @ RT)
7. Wash slides in dH2O, 5 mins then in 1xPBS, 5 mins
8. Block: ImmPRESS NHS 3 drops – 20mins
9. Add 130µl of the primary antibody (10 AB in PRIMARY DILUENT (PBS-Tx 0.3%))
10. Incubate at RT for 1HR or 4°C overnight
11. 1xPBS wash, 5mins
12. 20 antibody HRP ImmPRESS 3 drops – 45 mins
13. x3 1XPBS wash, 5 mins
14. Make ImmPACT DAB – 1 drop:1ml DAB buffer
15. Add ~3 drops DAB for X mins
16. dH2O wash, 5 mins
17. running dH2O wash, 5 mins
18. Place slides in Mayer’s haematoxylin (~2 mins) – depending on age of haematoxylin
19. Running tap water wash, 5 mins
20. Dehydrate tissue through ethanol: 70%, 90%, 100%, 100%, 5 mins each. Clear x 2 in Xylene, 5 mins and a final ~30 min xylene.
21. In fume hood: Coverslip with DPX mountant, leave to dry.