SS IHC

**Detection System:** SuperSensitive Polymer-HRP IHC Detection System (BioGenex, USA)

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

1. Dewax x2 in xylene, 5 mins each
2. Dehydrate through ethanol: 100%, 100%, 90%, 70%, 5 mins each. Wash slides in dH20 (5 mins)
3. Peroxidase quenching in 1:30 H2O2 to 1xPBS (10ml 30% H2O2:290ml PBS) (30 mins). Turn on the steamer approximately 17 mins 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 in the steamer for 20 mins, then cool in ice bucket (15 mins - until slides @ RT)
7. dH2O wash, 5 mins
8. 1xPBS wash, 5 mins
9. Add ~130µl of the primary antibody (10 AB in PRIMARY DILUENT (PBS-Tx 0.3%))
10. Incubate at RT 1HR or 4°C overnight
11. 1xPBS was, 5mins
12. Add ~130µl of Super-Enhancer reagent from SS kit, incubate RT, 20 mins
13. 1xPBS wash, 5 mins
14. Add ~130µl of the SS-label reagent from SS kit, incubate RT, 30 mins
15. 1xPBS wash, 5 mins
16. While the slides are in 1xPBS, make the DAB solution by adding 2 drops of the DAB chromogen per 1ml of DAB buffer, both from SS kit
17. Add ~130µl of DAB solution and leave for X minutes
18. dH2O wash, 5 mins
19. running dH2O wash, 5 mins
20. Place slides in Mayer’s haematoxylin (~2 mins) – depending on age of haematoxylin
21. running tap water wash, 5 mins
22. Dehydrate tissue through ethanol: 70%, 90%, 100%, 100%, 5 mins . Clear x2 in xylene, 5 mins and a final ~30 mins xylene.
23. In fume hood: Coverslip with DPX mountant, leave to dry