IHC protocol NOGGIN amplified (goat polyclonal anti-mouse Noggin AF719 R&D)

Day 1
Paraffin Sections
1. Dry slides Over Night in 37C Hand heat paraffin for 1hr at 55C to melt wax
2. Deparaffinize slides in 2 changes of xylene x 5min each
3. Rehydrate in graded alcohol 100% ➔ 95% ➔ 70%, 3min each
4. PBS wash 5min
5. Trypsin 6 min -7 min @37C
6. Wash PBS
5. Incubate slides in 3% H2O2 in methanol: 20min
6. PBS Wash 5 min
8. Block slides in 100mcl of TNB 30 min
9. Primary AB 1:100 in TNB 4C overnight

Day 2
10. TBST wash 3x 5min each
11. Secondary AB (1:300 anti-goat HRP OR 1:300 biotinylated anti goat AB) in TNB 1HR RT
12. TBST wash 3x 5min each and go to DAB (#15)
13. In Biotinylated AB use after wash : SA-HRP (diluted 1:100 in TNB) 30min RT
14. TBST wash2x 5min each
15. DAB Detection
   1. 5ml ddH2O
   2. 2 drops buffer ➔ VORTEX
   3. 4 drops DAB ➔ VORTEX
   4. 2 drops H2O2 ➔ VORTEX
20. Stop reaction in ddH20
21. Counterstain with Fastgreen 1 min ➔ Acetic Acid 30 sec ➔ ddH2O
22. Dehydrate in graded alcohol 70% ➔ 95% ➔ 100% ➔ Xylene
23. Coverslip