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IHC protocol SOX9 (rabbit polyclonal 5535 Milipore) (Eva Liu)

Day1

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. Antigen retrieval: trypsin 14 min 37C
6. Wash PBS
5. Incubate slides in 3% H2O2 in methanol: 20min
6. PBS Wash 5 min
8. Block slides TNB 60 min
9. Primary AB 1:2000 in PBS 2h in RT

Day 2

10. TBST wash 3x 5min each
11. Secondary AB 1:5000 anti-rabbit biotinylated in TNB 1HR RT
12. TBST wash 3x 5min
13. HRP 1:100 in TNB 30 min
14. TBST wash 3x 5min
15. DAB Detection: about 4 min
 1. 5ml ddH2O
 2. 2 drops buffer → VORTEX
 3. 4 drops DAB → VORTEX
 4. 2 drops H2O2 → VORTEX
16. Stop reaction in ddH2O
17. Counterstain with Fastgreen 1 min → Acetic Acid 30 sec → ddH2O
18. Dehydrate in graded alcohol 70% → 95% → 100% → Xylene
19. Coverslip