IHC protocol SOX9 (rabbit polyclonal 5535 Milipore)

**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. 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