Immunostaining Protocol

1. Rinse embryos in PBS
2. Remove Zona Pellucida in Acid Tyrode's solution (Millipore: MR-004-D | EmbryoMax Acidic Tyrode's Solution)
3. Wash thoroughly in PBS
4. Fix in 4% paraformaldehyde (freshly made in PBS with 0.1% Tween) overnight at 4˚C
5. Wash 3 times in PBS
6. Permeabilize for 20min at room temperature in 0.1-0.55% Triton X-100 in PBS
7. Wash 3 times in PBSX (PBS with 0.1% Triton X-100)
8. 4 x wash in PBX (since this moment all washes in PBX (PBS with 0.1% Triton-X))
9. 10 min in NH_4Cl (freshly prepared)
10. 3 x wash in PBX
11. Block in Blocking Buffer (10% FCS in PBS) between 40 min (RT) or longer to 6 h (can be O/N) in 4˚C
12. Pre-cool the solution. Primary Abs in Blocking Buffer overnight in 4˚C.
13. 4 x wash in PBX
14. Secondary Ab in Blocking Buffer 1 h 15 min in 4˚C. DO NOT keep it too long in 2˚AB. **Protect from light for ImmunoFluor staining protocol.**
15. 2 x wash in PBX
16. Nuclear staining –20-30min of 1:1000 Hoechst 33342 5mg/ml in PBX at RT
17. Wash twice in PBX (can be kept O/N) and twice in PBS
18. Mount in Prolong Gold (Invitrogen)

Example of Sox17 IF (using this protocol) in preimplantation mouse embryos: