

## **Whole Mount Staining of Vibratome Sections (to 700um)**

All steps should be done at 4oC while mixing

1. Following sectioning, block o/n in PBT + 10% goat serum.
2. Wash 1 hr with PBT + 1% goat serum
3. Stain o/n in 1o Ab in PBT +1%goat serum (use approximately 1ml per well in a 12 well plate).
4. Wash 5X 30 minutes in PBT +1% goat serum.
5. Stain o/n with 2oAb in PBT +1% goat serum.
6. Repeat 4.
7. Wash 30 minutes in PBS
8. Post-fix for 20 minutes at 4oC with fresh 4%PFA on 0.1MPB (1ml/well)
9. Wash 20 min in PBS (1-2ml/well)
10. Transfer sections to 6 well plate and wash o/n in 6-8 ml of PBS.
11. Clearing:  
30 min 20% glycerol in 1%KOH  
30 min 30% glycerol in 1%KOH  
30 min-1hr 50% glycerol  
1hr (until sinking) 70% glycerol  
Take pictures the same day

PBT: PBS with 1% TX100