Whole immunostaining of neurons by 3A10 antibody in cartilaginous fish (Turner et al. *Proc Biol Sci*, 2019)

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This is a modified method of the previous one published in O'Neill P, McCole RB, Baker CVH, *Dev Bio.* 2007

1. Fix embryos in 4% PFA at 4 degree overnight
2. Wash embryos in PBS with 1% triton (PBT-1) for 3 hours
3. Incubate them in 0.25% trypsin for 5 min. and immerse them in pre-cooled acetone in 10min.
4. Place embryos in 10% goat serum (GS), 1% dimethyl sulfoxide and 5% H2O2 in PBT-1, overnight. You will see air bubbles in the embryos.
5. Rinse embryos by PBT-1 three times by PBT-1
6. Dilute the 3A10 antibody (DSHB) at concentrations of 1:50 in PBT-1 containing 10% GS. Keep embryos at 4 degree for three overnights
7. Wash embryos by PBT-1 for an hour. Repeat 5 times.
8. Dilute Peroxidase-conjugated secondary antibody (Jackson laboratory) at a concentration of 1:500 in PBT-1 containing 1% GS.
9. Replace the solution by the second antibody/PBT-1 and incubate embryos for overnight.
10. Wash embryos by PBT-1 for an hour. Repeat 5 times.
11. Reaction was developed by transferring embryos to fresh DAB (0.5 mg/ml) activated with 0.003% H2O2 in PBT-1.