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Neurofilament staining of whole mount mouse embryos (from Behringer Lab)

Throughout the procedures, embryos should be gently agitated to improve tissue penetration. The protocol works best for E10.0 embryos. Punch holes in the forebrain and forth ventricle to help penetration.

1. Fix embryos in 4% paraformaldehyde in PBS (PFA) at 4C for 2 hours. Wash in PBT.
2. Transfer to 100% MeOH at –20°C O/N.
3. Bleach in 5:1 MeOH/30% H_2O_2 at RT for 3-5 hrs. Transfer to 100% MeOH and store at –20°C O/N.
4. Rehydrate embryos. 50%MeOH, 15% MeOH, PBS, at 4°C for 30 min each.
5. Incubate twice in PBSMT at RT for 1 hr each.
6. Incubate at 4°C O/N with anti-neurofilament antibody (2H3 monocloned, from Developmental Studies Hybridoma Bank) diluted in PBSMT (1:50, 20μl in 1ml).
7. Wash twice in PBSMT at 4°C and 3 times in PBSMT at RT for 1 hr each.
8. Incubate at 4°C O/N with secondary antibody (Peroxidase-conjugated Goat anti-Mouse IgG (Jackson ImmunoResearch Laboratories, Inc. 115-035-146), 1:500 diluted in PBSMT. (2 μl into 1ml of PBSMT)
9. Wash twice in PBSMT at 4°C and 3 times in PBSMT at RT for 1 hr each. Then wash in PBT at RT for 20 min.
10. Incubate embryos in 0.3mg/ml DAB (Sigma D-5905, carcinogenic!!!) in 0.5% $NiCl_2$ (10mg tablet in 34ml of 0.5% in $NiCl_2$) PBT at RT for 30 min or more.
11. Add H_2O_2 to 0.0003% (1/100 30% dilution and 1/1000 dilution) and incubate at RT until the color density looks good (usually ~10 min).
12. Rinse in PBT and dehydrate through a MeOH series: 30%, 50%, 80%, 100%, 100% for 30 min each.
13. Embryos may be cleared in benzyl alcohol: benzyl benzoate (1:2) (BABB). Note: polystyrene will dissolve in BABB, use glass containers.

PBSMT: 2% instant skim milk powder (10g), 0.1% triton X-100 (0.5ml) in 500 ml of PBS.

PBT: 0.2% BSA(Simga A-4378), 0.1% Triton X-100 in PBS.