Neurofilament Immunostaining of Mouse Embryos

(Rivera Lab)

(Adapted from R. Behringer's protocol)

Throughout the entire protocol, embryos should be gently agitated to improve the penetration of the tissue.

1. Fix the embryos in 4% parformaldehyde in PBS at 4 ºC for 2 hours. Wash in PBT.

2. Transfer to 100% methanol at -20 ºC overnight.

3. Bleach in 5:1 methanol/30% H_2O_2 at room temperature for 3-5 hours. Transfer to 100% methanol and store at -20 ºC overnight.

4. Rehydrate embryos. 50% methanol, 15% methanol and PBS at 4 ºC for 20 min. in each solution.

5. Incubate twice in PBSMT at room temperature for 1 hour each.

6. Incubate at 4 ºC overnight with anti-neurofilament antibody diluted in PBSMT (1:1000).

7. Wash twice in PBMST at 4 ºC and 3 times in PBSMT at RT for 1 hour each.

8. Incubate at 4 ºC overnight with secondary antibody diluted in PBSMT. For neurofilament: Peroxidase coupled goat anti-mouse IgG (1:200; Hyclone EA-1064-U (2 ml).

9. Repeat step 7, adding a final wash in PBT at room temperature for 20 minutes.

10. Incubate the embryos in 0.3 mg/ml DAB and 0.5% $NiCl_2$ in PBT at room temperatture for at least 20 min.

11. Add H_2O_2 to 0.0003% and incubate at RT until the color density looks good (usually ~10 minutes).

12. Rinse in PBT and dehydrate through methanol series: 30%, 50%, 80%, 100% for 30 min. each.

13. Embryos may be cleared in benzyl alcohol: benzyl benzoate (1:2) (BABB). Polystyrene will dissolve in BABB, so use glass containers.

PBSMT: 2% instant skim milk powder, 0.1% Triton X-100 in PBS.

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

DAB: Diaminobenzidine (Sigma D-5637) Carcinogenic.