

Immunohistochemistry on whole mouse embryos

This protocol is a combination of procedures used by Dent et al. (Development 105, 61-74, 1989) and LeMotte et al. (EMBO J. 8(1), 1989).

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

1. Collect embryos in PBS, dissecting away extraembryonic membranes.
2. Fix in methanol: DMSO (4:1) overnight at 4°C.
3. Transfer into methanol: DMSO: 30% H_2O_2 (4:1:1) for 4-5 hours at room temperature to bleach the embryos and block endogenous peroxidases. The embryos may then be stored for at least a few weeks in 100% methanol at -20°C.
4. Rehydrate the embryos. 50% methanol, 15% methanol, PBS, for 30 minutes each.
5. Incubate twice in PBSMT for 1 hour each at room temperature.
6. Incubate overnight at 4°C with primary antibody diluted in PBSMT (final dilution of antibody should be 1/1000).
7. Wash 2 times in PBSMT at 4°C and 3 times at room temperature for 1 hour each.
8. Incubate overnight at 4°C with the secondary antibody diluted in PBSMT. We use a 1/200 dilution of HRP coupled goat anti-rabbit IgG (Jackson ImmunoResearch, #111-035-003).
9. Repeat step 7, adding a final 20 minute wash in PBT at room temperature

HRP-coupled secondary antibody may be detected as follows:

10. Incubate embryos in 0.3 mg/ml DAB in PBT at room temperature for a minimum of 20 minutes. The color may be enhanced by adding 0.5% NiCl_2 to the DAB solution.
11. Add H_2O_2 to 0.03% and incubate at room temperature until the color density looks good, usually about 10 minutes.
12. Rinse in PBT and dehydrate through a methanol series: 30%, 50%, 80%, 100% for 30 minutes each.
13. Embryos may be cleared in benzyl alcohol: benzyl benzoate (1:2) (BABB). Note: polystyrene containers will dissolve in BABB.

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

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

DAB- Diaminobenzidine (Sigma D-5636) Carcinogenic