Immunohistochemistry protocol for cryostat sections
Inma Cobos 7/04

Fixation and cryoprotection:

1 Fix embryos overnight at 4 C in 4% paraformaldehyde, 0.1M PB.

2 Transfer embryos to 30% sucrose in 0.1M PB for several hours (after it sinks),

3 Transfer the embryos to well plates, suck the sucrose off and add OCT. Stir. Put the plate on the shaker at 4 C for 1 hour (stir them with a plastic pipette a few times to help the OCT embed the tissue). Transfer the embryos to plastic molds and freeze the blocks on dry ice. Blocks can be stored at -80 C several months if necessary.

4 Cut 20 μm sections on the cryostat and mount the sections on fisherbrand superfrost plus slides (No 12-550-15).

6 After sectioning put the slides into a clean slide box with some desiccant caps. Put the box in a plastic zip bag at –80 C.
Intracardiac perfusion with 4% Paraformaldehyde (PFA) in PBS and postfixation in the same fixative for 3 hrs
Cryoprotection with 30% sucrose for 1 day (after the brains sink).

Sectioning

4 Cut 10-20 μm sections on the cryostat and mount the sections on fisherbrand superfrost plus slides (No 12-550-15).

6 After sectioning the slides can be put into a slide box with some desiccant caps and stored (in a plastic zip bag) at –80 C.

DAY 1

Immunohistochemistry

3x5' PBS
1x10' 0.2% Triton X-100 (Sigma) in PBS (PBS-Tx 0.2%)

Endogenous Perioxidase Blockade (PROTECT FROM THE LIGHT WITH ALUMINIUN FOIL)

1x20' incubation in:
- 0.5% Hydrogen peroxide in PBS-Tx 0.2%

3x5' PBS-Tx 0.2%

Blockade Nonspecific Binding

60' incubation in:
- 10% Normal **Goat** Serum (Vector), 0.2% gelatin, 2% non-fat milk, in PBS-Tx-0.2%

1 day Primary Antibody Incubation (4 Degrees)

- **Antibodies:**

- 3% Normal **Goat** Serum (vector), 0.2% gelatin in PBS-Tx 0.2%

DAY 2

3x5' PBS-Tx 0.2%

Secondary Antibody Incubation (4 Degrees)

1-2h incubation in:
- **1:200 Goat anti-Rabbit (Mouse or rat)** biotinylated antibody (Vector)
- 3% Normal Goat Serum (Vector), 0.2% gelatin, in PBS-Tx 0.2%

3x5' PBS-Tx 0.2%

*** ABC preparation 30-45 before use

Avidin-Biotin Complex (ABC Vector), (Room temperature, slow shaking)

60' incubation in:
- **1:300 ABC** in PBS-Tx 0.2%

3x5' PBS-Tx 0.2%
1x 5' Tris buffer, 0.05M, ph 7.5

Development in diaminobenzidine (DAB)

DAB solution:
- one tablet of DAB (sigma D-4293) in 5 ml of water

Add to filtered DAB solution:

0.01% Hydrogen peroxide (3.3ul of 30% H_2O_2 per 10 ml DAB solution)

2x5' washes Tris Buffer,

dehydrate, immerse in Xilene and cover.

Or,

Secondary Antibody Incubation (4 Degrees)

1-2h incubation in:

- **1:300 Goat anti-Rabbit (Mouse or rat)** fluorescent antibody (Molecular Probes)
- 3% Normal Goat Serum (Vector), 0.2% gelatin, in PBS-Tx 0.2%

3x5' PBS-Tx 0.2%

counterstain and cover