

NADIA Scientific Core IHC Perfusion Protocol

Materials

- Perfusion Pump –Cole Palmer Masterflex L/S Model #7520-10
- 3.1mm I.D. tubing (Cole Palmer # 06409-16)
- 20 gauge needle blunted for mice (For rats, a small/mouse sized feeding needle)
- Scissors, forceps, hemostats, clamps, syringes
- Large metal tray with grate on top (collect para and blood)
- Small jars with lids for brains
- Pentobarbital sodium (100 mg/kg)
- 0.1M phosphate buffered saline (PBS, pH7.4)
- 4% Paraformaldehyde (PFA)

Flow rate for pump

Rat: 20ml/min for 200-300 gram body weight;

Adjust flow rate as appropriate for the body weight, e.g. 100-150 gram rat, approximate 10-12 ml/min flow rate.

Procedure

Animals are firstly i.p. injected with 100mg/kg pentobarbital sodium.

1. After sedation, animals are checked for toe-pinch reflex to check for pain reflex before any procedures are done. Once sedated put on the top of grate.
2. Following complete anesthesia, the animal is cut open below the diaphragm and the rib cage is cut rostrally on the lateral edges to expose the heart.
3. A small hole is cut in the left ventricle and the needle is inserted into the aorta and clamped, then the right atrium is cut to allow flow.
4. The animal is transcardially perfused with PBS wash for 4-5 minutes or until liver is cleared of blood. Visualize the heart and liver as perfusion commences. The right ventricular chamber should remain somewhat darkened in color when compared to the left ventricular chamber. The liver should begin to blanch as blood is replaced with PBS.
5. Next perfuse with 4% Paraformaldehyde for 4 minutes. Visualize the animal's extremities for evidence of tremors resulting from the aldehyde-crosslinking of nerves and muscle. This is an indication that fixation is taking place.
6. Remove head (decapitate at a level even with forelimbs to ensure that the entire brainstem is included), trim off skin and store head in small jar of 4% Paraformaldehyde for 24 hours.
7. After 24 hours, transfer head to PBS and store in 4°C.

* 4% Paraformaldehyde (PFA)

- Heat 500 ml ddH₂O to 60°C
- Add 40 g paraformaldehyde and stir (stir for about 15 minutes in the fume hood.)
- Add several to many drops (10 drops) of NaOH while stirring, until clear
- Add 500 ml of 0.2 M phosphate buffer (Na₂HPO₄ 6.72 g; NaH₂ PO₄ 20.41 g; dd H₂O 1000 ml, mix to dissolve and adjust pH to 7.4)
- Filter the solution and use 24 hours