

Crews MRI (Prohance) 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

Pentobarbital

Large metal tray with grate on top (collect para and blood)

Heating pad (animal is kept on heating pad during perfusion to maintain body temperature). *The Crews lab uses a heated pad that pumps warm water (<http://www.hallowell.com>) hooked up to a rectal thermometer. The pad needs to be water-proof. You can also get a pad that is heated in a water bath or microwave and holds heat at 37°C (<http://www.amazon.com/Pet-Supply-Imports-SnuggleSafe-Heating/dp/B00008AJH9> or <http://www.braintreesci.com/Products/39dp.asp>).*

Solutions

Saline wash- 1:10 solution of Prohance in 0.9% saline, **warmed to 37° C.**

Fixative- 1:10 solution of Prohance in 10% formalin, buffered, pH 7.4 and room temperature

10% formalin, buffered (such as catalogue # 23-245-685 from Fisher Scientific)

Storage- 1:100 Prohance in PBS

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

1. Anesthetize the animals with 100mg/kg pentobarbital i.p. or other suitable anesthetic.
2. After sedation, animals are checked for toe-pinch reflex to check for pain reflex before any procedures are done. Once sedated put on heating pad (and insert temperature probe if you are using a self-regulating heating system).
3. 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.
4. 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.
5. The animal is transcardially perfused with warmed saline-Prohance wash for 4 minutes 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 saline.
6. Next perfuse with room temperature fixative (formalin-Prohance) 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.
7. 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 10% buffered formalin (with no Prohance) for 24 hours.
8. After 24 hours, transfer head to 1:100 solution of Prohance in PBS and store in 4° C.