UC Davis/NIH NeuroMab Facility
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Transcardial Perfusion of Mice for Immunohistochemistry

Work in a fume hood using a perfusion stage and collection pan that will collect the perfusate (*i.e.*, any formaldehyde-containing solutions) and allow for its proper disposal as per institutional guidelines. This protocol is for use on mice weighing between 15 g and 25 g.

Anesthesia.

1) Weigh the mouse to calculate accurate anesthetic dosage and administer appropriately (*e.g.*, 100 mg/kg Sodium Pentobarbital in Fatal Plus Solution, Vortech Pharmaceuticals) *via* intraperitoneal injection.

2) Check for complete anesthetic state (*e.g.*, loss of corneal reflex by lack of blinking when air is blown into eyes and loss of pedal pain reflex by lack of movement of paw/tail when squeezed).

3) The mouse should be completely anesthetized after ≈10 min. If the mouse still reacts to eye blink/pedal reflex after 10-15 min, then administer additional anesthetic (*e.g.*, 100 mg/kg Sodium Pentobarbital in Fatal Plus Solution). Note the degree of reaction observed and repeat every 5 min until complete anesthesia is achieved.

Surgery/Perfusion.

4) Place the mouse on perfusion stage in collection pan and pin limbs to allow for exposure of peritoneal cavity.

5) Use forceps in one hand to grab the skin over the xiphoid process and use scissors in the other hand to cut, parallel to the spine, a patch of skin to reveal the outer abdominal wall.

6) The xiphoid process should now be visible. Use forceps in one hand to lift it, and use scissors in the other hand to cut into and through the abdominal wall laterally, taking care to avoid cutting any organs or major vessels.

7) The diaphragm should now be visible. Cut through diaphragm laterally, taking care to avoid cutting any organs or major vessels.

8) Cut through the ribs and parallel to the lungs to create a chest "flap".

9) Retract chest "flap", fold it over the head and clamp it in place with a hemostat.

10) Use forceps in one hand to grasp the heart near its apex, use scissors in the other hand to make an incision into the left ventricle and insert into the incision a 25 gauge needle attached to a peristaltic pump *via* silicon tubing.

11) Clamp needle in place using a small hemostat.

12) Begin rapid perfusion of 1X PBS perfusate containing heparin (recipe below). Turn on peristaltic pump at a rate of 7 mL/min (*e.g.*, 25 rpm on a Watson Marlow 323 peristaltic pump with 1.6 mm I.D. silicon tubing, I.V mini drip set) and begin perfusion of 11 mL (≈2 min) of ice-cold (4°C) 1X PBS perfusate. Once perfusion has begun, cut the right atrium to allow drainage.

13) Switch perfusate to ice-cold (4°C) 4% formaldehyde (recipe below) and perfuse 25 mL at 5 mL/min (≈5 min). The upper body of the mouse should contract following the switch to fixative; this is a sign of the correct needle position.

14) After perfusion of the mouse is complete, perform guillotine decapitation just behind the ears and remove the skin and bone to expose the brain, taking care to minimize damage to tissue.

15) Remove the skull by chipping away with bone rongeurs.

16) Insert a spatula between the ventral side of brain and the bottom of skull to cut nerves and scoop out the brain, taking care to clear away meninges and avoid slicing through the tissue, especially near the olfactory bulbs.

Cryoprotection.

17) Place fixed brain in a 50 mL conical tube containing ice-cold (4°C) 10% sucrose solution (recipe below) and incubate at 4°C overnight. The brain should equilibrate completely, as evidenced by its sinking to the bottom of the tube.

18) Replace 10% sucrose with ice-cold (4°C) 30% sucrose and allow the brain to equilibrate completely at 4°C, as evidenced by its sinking to the bottom of the tube. This may take as long as three days.

19) Hemisect the brain by cutting through the midline with a fresh blade (razor or scalpel). Flash freeze by placing hemisphere medial side down on a block of dry ice and covering with pulverized dry ice.

20) Section immediately on a freezing stage microtome or wrap frozen hemispheres in plastic wrap, then in aluminum foil and store at -80°C until sectioning.

Recipes

10X PBS Stock Solution (1 L)

2.3 g	KH_2PO_4	17 mM (MW 136.09)
7.4 g	Na_2HPO_4	52 mM (MW 141.96)
87.7 g	NaCl	1.5 M (MW 58.44)

Allow reagents to dissolve into ≈900 mL of ddH_20, adjust pH to 7.4 and bring to final volume of 1 L.

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1X PBS Perfusate (1 L)

1) Dilute 100 mL of 10X PBS Stock Solution into 900 mL of ddH_20.
2) Adjust pH to 7.4 with concentrated NaOH or HCl as needed.
3) Vacuum-filter solution through Whatman #2 paper over a Buchner funnel.
4) Add 10,000 units of heparin (*e.g.*, ≈54 mg of 185.8 units/mg stock, Akron Biotech) and mix.
5) Chill to 4°C and keep on ice when in use.

0.4 M PB Stock Solution (2 L)

91.37 g Na_2HPO_4	320 mM (MW 141.96)
20.98 g $NaH_2PO_4 \cdot H_2O$	76 mM (MW 137.99)

Allow reagents to dissolve into ≈1600 mL of ddH_20, adjust pH to 7.4 and bring to final volume of 2 L. For 0.2 M (fixative solution) or 0.1 M (sucrose solutions), dilute accordingly in ddH_20 and check that pH is 7.4.

Freshly Prepared 4% Formaldehyde (from Paraformaldehyde Powder) Fixative Solution (1 L)

1) Heat ≈400 mL of ddH_20 to ≈60°C on a heated stirring plate in chemical fume hood (do not exceed 65°C as this will cause degradation of formaldehyde).
2) Add 40 g of paraformaldehyde.
3) Slowly add 10N NaOH dropwise and stir until solution becomes clear. Monitor pH, which should not exceed pH 10, as this will cause degradation of formaldehyde.
3) Add 500 mL of 0.2 M PB.
4) Adjust pH to 7.4.
5) Vacuum-filter solution through Whatman #2 filter paper over Buchner funnel.
6) Adjust final volume to 1 L with ddH_20.
7) Chill to 4°C and keep on ice when in use.

Sucrose solutions (100 mL)

1) For a 10% sucrose solution, dissolve 10 g of sucrose in ≈70 mL of 0.1 M PB. For a 30% sucrose solution, dissolve 30 g in ≈70 mL of 0.1 M PB.
2) Check that pH is 7.4 and bring to final volume of 100 mL with 0.1 M PB.
3) Store at 4°C.

References: *Rhodes et al., J Neurosci 15:5360, 1995 (https://www.ncbi.nlm.nih.gov/pubmed/7623158);*
Manning et al., PLoS One 7:e38313, 2012 (https://www.ncbi.nlm.nih.gov/pubmed/22675541);
Bishop et al., J Neurosci 35:14922, 2015 (https://www.ncbi.nlm.nih.gov/pubmed/26538660).