TissueVision Standard Perfusion

Prepare ketamine/xylazine cocktail:

- Ketamine/xylazine cocktail:
  - ketamine 100mg/kg
  - xylazine 10mg/kg
  - Made in PBS or physiological saline

Prepare hood for perfusion

- Turn on peristaltic pump and set rate to 10ml/min
- Prep surgical tool
- Prime the PFA and PBS tubes
- Continue to prime the tubing to the blunt needle with PBS

Anesthetize mice with ketamine/xylazine cocktail:

- Route: IP
- Dose: 0.01mL/gm body weight
- Once animals appear lethargic, perform a toe pinch test.
  - **If after 10 minutes**, mice respond to toe pinch test, administer a redose at ¼ dose of the ketamine/xylazine cocktail.
  - ***If there is no reflex response***, begin perfusion

Perform Perfusion

1. Secure the animal on a flat surface to begin transcardial perfusion
2. Using straight edge scissors, cut the chest cavity open right beneath the Xiphoid Process
3. Cut into the diaphragm to expose the heart and lungs
4. Clamp the Xiphoid Process down and pull the clamp up over the head, laying the clamp down flat so the chest cavity is exposed
5. Remove part of the rib cage if you do not fully see the heart
6. Take the blunt needle tip of the perfusion tube and orient it towards the apex of the heart
7. Insert the needle through the apex of the heart, keeping the needle in the left ventricle
8. Secure the needle so it does not move, or hold it steady so the needle does not come out
9. Using the curved edge scissors, create an incision in the Right Atrium of the heart
10. **Once blood is visibly flowing from the heart, turn on the perfusion pump**
11. Perfuse **50ml PBS** at a rate of **10mL/minute**.
    1. During this process, you should see the liver lose blood and turn a tan color. This will indicate a good perfusion.
12. Perfuse **50ml 4% PFA** at a rate of **10mL/minute**.
    1. During this process, the extremities will become stiff, and potentially twitch.
13. Remove the animal from the perfusion area - the entire mouse body should be rigid now.

Removing the brain

14. Cut off the head with a large pair of scissors
15. Peel back the skin and muscle on the top of the head to expose the skull
16. Using the straight edge scissors carefully cut down the sagittal suture starting from the interparietal bone.
17. With tweezers, gently peel away the parietal bones. .
18. Gently cut away and break apart the interparietal and occipital bones
19. Gently cut the optic nerves
20. With a flat spatula, place under the olfactory bulbs. Simultaneously push up and out to the remove the brain

Brain Storage

21. Place brain in 4% PFA at 4C for 12 to 24 hours.
22. Change solution to PBS and 0.1% sodium azide for storage and shipment.

Buffers

1X PBS

Product: 10X Phosphate Buffered Solution

Company: Fisher Scientific

Cat #: BP399-1

- To make **1 liter of 1x PBS:**
  - dilute 100 ml of 10X PBS in 900 mL of distilled water

4% PFA

Product: Paraformaldehyde 16% Solution

Company: Electron Microscopy Sciences

Cat #: 15710-S

- To make **1 liter of a 4% PFA solution**:
  - dilute 250 mL of 16% PFA into 750 mL of 1X PBS

0.1% Sodium Azide in PBS

Product: 5% (w/v) Sodium Azide

Company: Ricca Chemical Company

Cat #: 7144.8-16

- To make **1 liter of 0.1% Sodium Azide in PBS**:
  - dilute 200 mL of 5% Sodium Azide in 800 mL of 1X PBS