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# Mouse Perfusion Protocol

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol lists materials, setup, and steps to perform a fast and successful mouse perfusion for brain harvesting.

## Materials

**Equipment**

- Peristaltic Pump (Watson-Marlow 205S)
- Isoflurane vaporizer with induction chamber and nose-cone
- 4 Beakers
- Styrofoam box and lid
- Ice container

**Tools**

- 2 Surgical Scissors, large and small
- 1 Hemostat
- 2 Forceps, blunt and sharp
- 1 Spatula

**Solutions**

- PFA (4%PFA/PBS BRAND, ~40 ml per animal)
- PBS (1x, ~20 ml per animal)
- 1x PBS with $NaN_3$ (0.02% NaN3, enough to fill vials)
- Isofluorane (brand, cas #)
- $O_2$ tank

**Consumables**

- Parafilm
- Aluminum foil
- 26g needles
- Needles for pinning (18g)
- Bench Pads
- Ice
- Sample vials (for each brain) - brand

## Safety warnings

paraformaldehyde is toxic, work in a chemical hood

## Ethics statement

the protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee. This work was approved by UCSD IACUC S19028

## Setting up

10m

1

**Beakers and Solutions**

- Prepare the first beaker with cold PFA, on ice, cover with parafilm (leave small opening for the tube)
- Prepare the second beaker with cold PBS, on ice, cover with parafilm (leave small opening for the tube)
- Prepare a third beaker for waste collection (end of the line)

**Peristaltic Pump (using one line)**

- Set up the peristaltic pump to a rate of 12 mL per minute (50 RPM)
- Purge the line with PBS by setting it to **CW** (Clockwise) and press **start**
- When the line is purged press **stop**
- Place a 26G needle on the end of the tubing

**Tubes**

- Label, fill tubes with PFA and put on ice, ready for brain harvesting

**Work station**

- A convenient workstation is a styrofoam surface (wrapped in aluminum foil) for pinning the mouse, placed at an angle to direct the exit flow of into a waste container (e.g. make a hole/opening towards the bottom of the styrofoam surface to guide the waste into a collection beaker placed inside a container onto which the surface rests
- Place bench padding where brain dissecting will be performed under the dissection tools, for easy cleanup
- Set up anesthesia induction chamber with 1-3% isofluorane in oxygen using a vaporizer

## Perfusion and Harvesting

12m

2 **Sedating Mouse**

1. Sedate mouse in a chamber
2. Observe respiratory rate to evaluate the level of anesthesia
3. Use toe-pinch and corneal reflex to ensure mouse is on a stable surgical plane
4. Switch isoflurane flow from chamber to nosecone
5. Move the mouse over to the perfusion station in nosecone
6. Double-check toe-pinch
7. Pin down the mouse

3 **Exposing Heart**

1. Grab the sternum with forceps (blunt, left hand) and make a v-cut incision below scissors (right hand), exposing the diaphragm
2. Make 2 upward cuts (left and right side of the body) through the diaphragm, ribcage, and skin
3. Clamp the sternum with a hemostat and open the chest to expose the heart

4 **Perfusion**

**Ensure that the tubing has been purged with PBS and that there is a 26G needle on the end**

1. **Start** pump (running PBS, **CW**)
2. Control the heart with forceps (blunt, left hand) and insert the bevel of the needle in the left ventricle
3. Immediately make a small incision in the right atrium with the sharp forceps
4. Perfuse with PBS for 1 min (Make sure to hold the needle steady, and not go too deep into the heart)
5. **Stop** the pump and switch the line from the PBS to the PFA beaker
6. **Start** the pump perfuse with PFA for 3 min (In case of successful perfusion: the organs/paws will immediately start getting paler and the mouse will display muscle twitches)
7. Press **CCW** (Counterclockwise) to remove all PFA from the line back into the beaker *(and start dissection of section 4)*
8. **Stop** the pump
    - If another dissection follows: place it in PBS press **CW** and **Start** to purge the line and press **stop**
    - If the last dissection: Follow the same steps to purge the line with PBS and dry by passing air through the line

**Ensure proper disposal of liquid chemical waste**

5 **Brain Dissections**

*Perform during step 8 of section 4*
Ensure excess PFA has run off mouse into collection beaker

1. Move the mouse to the lab pad for dissection
2. Use large surgical scissors to decapitate the mouse
3. Dispose of carcass in institute appropriate waste bag (Ensure proper disposal of PFA contaminated carcass)
4. Use smaller surgical scissors, forceps, and spatula to cleanly dissect the brain
5. Place brain in labeled vial with PFA solution on ice
6. Move vials with brains into the fridge with gentle shaking overnight

## Brain Perseveration

1h 30m

### 6 Wash and storage

- The next day, wash harvested brains 3x for 30 minutes in 1x PBS with Aza (0.02% $NaN_3$)
- The brains can be stored at 4°C