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Mouse Perfusion Protocol V.1

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

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Disclaimer

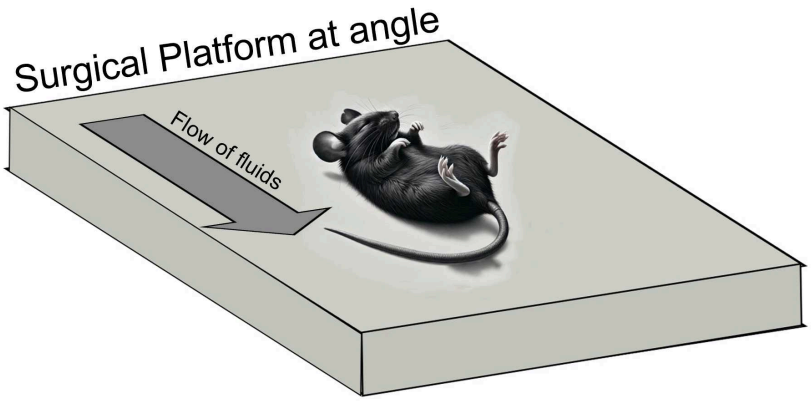
Abstract

Protocol for perfusing mice with fixative, including description of creating all required solutions.

Materials

Equipment:

Surgical Platform



Surgical platform at an angle that allows for flow of fluids off platform waste disposal.

Equipment	
Surflo winged infusion set 21G 3/4	NAME
Terumo	BRAND
TER 3SV-21BLK	SKU

Equipment

Insulin gauge needle (BD Ultra-fineTM II, NJ, USA)	NAME
(8 mm x 0.3 mm)	TYPE
BD	BRAND
328838	SKU
https://www.bd.com/en-sg/offerings/diabetes-care/insulin-syringes/bd-insulin-syringes-with-bd-ultra-fine-needle-8mm-x30g	LINK
0.3 mL	SPECIFICATIONS

Equipment

Variable-Flow Peristaltic Pump	NAME
Fisherbrand	BRAND
13-876-2	SKU
https://www.fishersci.com/shop/products/fisher-scientific-variable-flow-peristaltic-pumps-4/p-158215	LINK

Reagants:

- PBS Tablets, Phosphate Buffered Saline, Fisher BioReagents **Fisher Scientific Catalog #**BP2944100
- Paraformaldehyde Powder (PFA) **Catalog #**P6148
- Sodium Hydroxide **Fisher Scientific Catalog #**BP359500

1000ml of 4% PFA solution preparation:

1. Measure 600 mL of ddH2O in a graduated cylinder, and heat it to 70 °C to 90 °C (note: not directly heating with

buffer).
2. Weigh 40 g of Paraformaldehyde Powder (PFA) **Catalog** #P6148 , and dissolve it in the above-mentioned solution that is continuously heated, stir, and add an appropriate amount of NaOH (about 0.1 g for 1000 mL) to aid the PFA dissolving.
3. Add 10 tablets of
PBS Tablets, Phosphate Buffered Saline, Fisher BioReagents **Fisher Scientific** **Catalog** #BP2944100
4. Make the volume to 1000 mL .
5. Adjust the PH value to
pH 7.2 - pH 7.4 (< 0.3 g Sodium Hydroxide **Fisher Scientific** **Catalog** #BP359500 , need not adjust the PH).
6. Filter with

Equipment

Millipore® Stericup® Quick Release Vacuum Filtration System	NAME
0.22 um polyethersulfone membrane filtration system	TYPE
Millipore	BRAND
All Photos(3) Key Documents COA/COQ View All Docum	SKU
https://www.sigmaaldrich.com/US/en/product/mm/s2gpu05re	LINK
0.22 um filter	SPECIFICATIONS


7. Bottle and store at 4 °C until use.

Troubleshooting

Safety warnings

Paraformaldehyde (PFA) and sodium hydroxide are hazardous substances that can be dangerous if not handled safely. Waste includes previous hazardous chemicals, as well as potential biological hazards. Handling should only be done with proper peronsal prote

Ethics statement

Experiments involving animals must be conducted according to internationally accepted standards. Users should always have prior approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s). Prior ethics approval should be obtained before performing these experiments.

Before start

Ensure the animal is under proper anesthesia plane per local University and IACUC standards.

Perfusion Steps

1 Rinse the peristaltic pump thoroughly with saline or PBS for more than 5 minutes

2 Start the peristaltic pump with normal saline/PBS, make sure everything is well connected (pump, tube, needles, saline/PFA), and there are no air bubbles in the tube.

3 After anesthesia, the animals are fixed tightly with pins on four paws. Open the abdominal
and thoracic cavity with forceps in the left hand and scissors in the right hand. Remember that the tips of scissors are as close as possible to the bone to reduce the possibility of cutting the blood vessels.

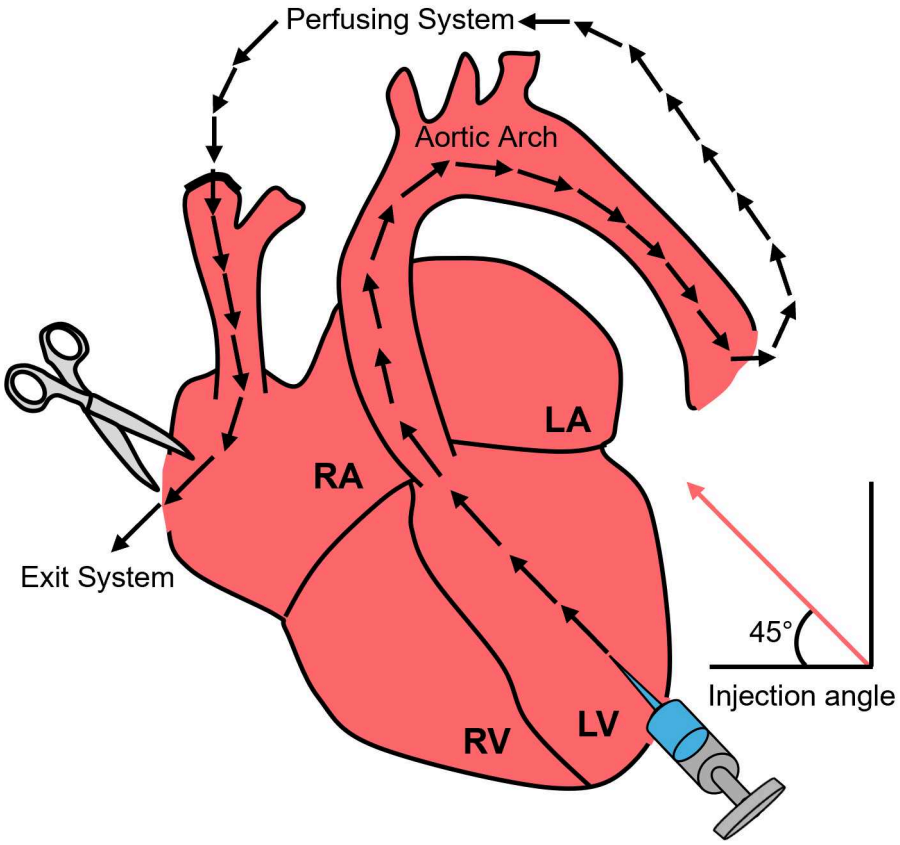
Safety information

Anesthesia for euthanasia must follow and abide by local IACUC and University standards. Ensure proper anesthesia plane before performing perfusion.

4 Cut a small hole in the right atrium, inject saline with a needle connected to a tube in the pump into the left ventricular, and you will find blood flowing out of the right atrium.

Note

The angle of injection should be 45 degrees towards the Aortic Arch



5 Perfuse with PBS for less than 5 minutes and stop the pump when the saline running out of the right atrium is clear (no blood is found). Change the tube from a saline bottle to a 4% PFA bottle (stop the pump first and change the tube second to reduce the possibility of bubbles in the tube). Open the pump again and perfuse animals with 4% PFA for another 10 minutes.

Note

Optimal flow settings for listed Peristaltic Pump (below) is between 32-25.

Equipment

Variable-Flow Peristaltic Pump	NAME
Fisherbrand	BRAND
13-876-2	SKU
https://www.fishersci.com/shop/products/fisher-scientific-variable-flow-peristaltic-pumps-4/p-158215	LINK

Note

Proper perfusion will result in tail rising upwards upon PFA perfusion.

Safety information

Paraformaldehyde (PFA) is a hazardous substance that can be dangerous if not handled safely

5.1 10 minutes for adults and 7 minutes for younger than P10.

6 Dissect the brain, and keep the tips of scissors as close as possible to the bone. This will reduce the damage to the brain tissue. Post fixed tissues in 4% PFA for less than 1 hour or in PFA overnight.

Safety information

Dispose of waste in accordance with local EHS standards.

Acknowledgements

This protocol was adapted from Mei Lab Perfusion, staining and stock protocol.