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Paraformaldehyde Animal Perfusion for Fixed Tissue Extraction

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SOX6 mDA differentiation



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Protocol status: Working

We use this protocol and it's working

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Abstract

We used this protocol to fix the brains of grafted mice. The result was a correctly fixed animal tissue.

Materials

50 ml Ice-cold PBS1X

50 ml Paraformaldehyde 4%

Pump

27g butterfly needle with a needle stop until bevel.

Protocol materials

 Formaldehyde 4% stabilised, buffered (pH 7.0 $\pm$ 0.1) **VWR International (Avantor) Catalog #9713**



Pre-perfusion Preparation

- 1 Prepare the surgical area for Paraformaldehyde, for example, tissues that will be discarded as toxic or PFA Neutralizing Absorbent Pads.
- 2 Prepare a 50 ml Falcon with PBS1X and another with PFA 4%. Maintain  On ice
- 3 Program the pump to start at 10 ml/min.
- 4 Make a needle stop at the bevel of the needle and install the needle on the pump tube.
- 5 Run some water to clean the tube
- 6 Run PBS1X so the tube is filled with PBS1X and ready to use.
- 7 Prepare your surgical tools
 - Blunt-end scissors
 - Blunt forceps
 - Sharp-end small scissors
 - Surgical mouse silicon pad

Animal Euthanasia

- 8 Induce anesthesia on mouse using isoflurane soaked tissue inside a 50 ml Falcon by placing its head inside until movement stops and breathing slows. 

Animal Euthanasia

- 9 Inject 100 mg/kg pentobarbital IP to mouse, ensuring narcotic overdose. Ensure terminal anesthesia by lack of pedal reflex (paw retracts when pinched). The animal should be not fully dead. 
Alternatively, use ketamine 100 mg/kg.

Surgery

- 10 Place the animal on the surgery pad and position the limbs away from the chest.
Using needles to pin the animal onto the pad is a classic step, but we used surgical tape to stick onto the PFA neutralizing pad place on top of the silicon surgical pad.
- 11 Using blunt-end scissors and blunt forceps, cut the skin and muscular fascia of the lower abdomen, lower than the rib cage. You will expose the liver and the diaphragm
- 12 Carefully move the livers to better access the diaphragm and the ribs. With the scissors, cut the diaphragm and the rib by the sides with care not to cut heart or lungs. This will cause a depressurization of the lungs and the heart will enter tachycardia to compensate.

Intracardiac Perfusion

- 13 Make sure the 27G butterfly needle has ice-cold PBS1X. Make sure that the needle has a needle stop around the bevel to avoid trespassing the cardiac walls.
We use the needle cover to cut up to the bevel, since mice have very small hearts. Just exposing the bevel was enough.
- 14 Cut the right atrium of the heart with the small sharp scissors, so injected volume has an escape. Blood will pour profusely.
- 15 Inject the PBS1X needle into the left ventricle and start the pump at 10 ml/min.
In our case, it was a Agnethos' Peristaltic Pump (120U) at 25 rpm. Check beforehand which rpm is necessary to reach 10 ml/min.
- 16 Exsanguinate animal with 50 ml PBS1X. The liver should lose their red colour and turn to a yellowish-fleshy colour.
- 17 Change to 50 ml PFA 4%. The muscles will start fixing so the animal will start moving.
The animal is not alive.
In our case we used Formaldehyde 4% stabilised, buffered (pH 7.0 ±0.1) (VWR Chemicals, #9713)
- 18 Stop the pump and remove needle.
- 19 Extract tissue as desired.



Formaldehyde 4% stabilised, buffered (pH 7.0 ±0.1) **VWR International**
(Avantor) Catalog #9713



- 20 If continuing with another animal, rinse tube with water and re-fill with PBS1X. Otherwise, clean and discard waste as your local policies.