Transcardial Perfusion in Mouse

In 1 collection

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ABSTRACT

This protocol details about the transcardial perfusion in mouse.

ATTACHMENTS

338-741.pdf

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

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MATERIALS

Materials Needed:

- Aluminum-wrapped Styrofoam
- Plastic lid (to capture runoff)
- Labmat (on bench and on styrofoam)
- 30 mL syringe with butterfly needle with cut tip

*Fill with 30 mL PBS and push PBS through tubing so all air bubbles are expelled.

- Perfusion pump: 100-120 mL/hour

Dissection tools

- 1 tube with 2 small drops heparin for blood
- 1 tube for tail
- 2 conicals with ethanol or formalin fixative
- 30 mL /mouse [M] 0.1 Molarity (M) PBS + heparin (1:100)
- 70% ethanol bottle

4% PFA in 0.1 M NaPO4 (1L)

Dissolve into 500ml diH20:

- 11.36 g sodium phosphate dibasic
- 2.76 g sodium phosphate monobasic

Once dissolved:

- Add 40 g PFA (powder)
- Fill to 1 L with diH20

Transcardial Perfusion

1 Make mixture of ketamine:xylazine:acepromazine (4:2:1) sufficient for anesthesia of all mice (~ 30 µL / 20 g mouse). Record ketamine used in controlled substance log book.

2 Apply anesthesia to one mouse via intraperitoneal injection, and place mouse in bucket long enough for anesthesia to take effect. Apply a hard toe pinch until mouse no longer reacts, ensuring that the mouse can no longer feel pain before proceeding.

3 Place mouse, abdomen-up, on Styrofoam block wrapped in lab mat. Spray mouse abdomen with 70% ethanol. Grasp skin below ribcage with forceps and cut skin with scissors from middle up either side towards the armpits, cutting through ribcage.

Note

Avoid blood vessels and organs. Diaphragm should carefully be cut circumferentially.

4 Remove pericardium and peripheral fat to expose heart. Hold back ribs with hemostats.

Note

* At this point blood can be taken via syringe from the right ventricle if desired. 200 µL to 300 µL should be sufficient.

5 Place blunted butterfly needle into left ventricle and cut right atrium. Push PBS through syringe by using perfusion pump at 2 mL/minute rate.

Note

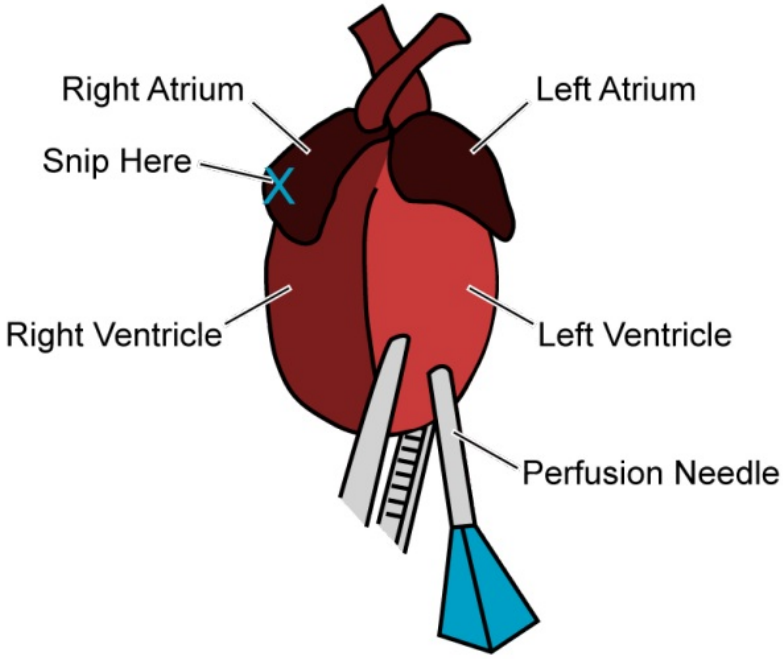
Liver should lose pigment and effluent should be dark blood then become more PBS as perfusion continues.

6 After perfusion is complete, remove brain, spinal cord and other desired parts and transfer to fixative.

Note

* Brain can be removed by carefully drilling hole with scissors at olfactory bulb junction and spreading scissors apart so skull splits in half.

Perfusion Schematic



Perfusion Solutions

7 Weigh PFA under the hood and using bench pads.

8 Heat and stir (in chemical hood).

9 Lets dissolve the PFA around 60 °C (use the thermometer). Do not let the temperature exceed 65 °C (remove from hot plate temporarily if necessary).

10 Once dissolved but still foggy, pH 7.4 with NaOH (it should make the PFA clear).

11 Prepare a big filter funnel using Whatman filter paper.

12 Filter the 4% PFA into a liter bottle/w lid.

13 Store the PFA covered at 4 °C.