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Transcardial perfusion of mouse tissues

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We use this protocol and it's working

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Abstract

This protocol describes how to perform transcardial perfusion and fixation of mouse brain tissues in preparation for immunohistochemical staining or histology. This process includes lethal overdose of mice with sodium pentobarbitone, occlusion of the descending aorta, followed by transcardial perfusion with phosphate buffered saline to clear blood from the vasculature and paraformaldehyde to fix mouse brain tissues.

Attachments

it73bj7ap.docx

97KB

Materials

Equipment:

- Peristaltic pump, tubing and gavage
- Surgical tools – haemostatic forceps, scissors, pins

Consumables:

- 5 mL , 50 mL sample containers
- 1 mL syringe

Key reagents:

- Sodium pentobarbitone
- Isopentane
- Paraformaldehye (PFA)

Solutions:

- 10x PBS

1. 77.3 g of NaH2PO4.H2O ([M] 0.28 Molarity (M)), 203.7 g of Na2HPO4 ([M] 0.72 Molarity (M)), 177.4 g of NaCl ([M] 1.5 Molarity (M)) in 2 L dH2O, pH 6.9

- 1x PBS, pH 7.4

1. 100 mL of 10x PBS in 900 mL dH2O, no pH adjustment required

- 4% PFA in 1x PBS pH 7.4

1. See related protocol – Preparation of 4% paraformaldehyde solution for transcardial perfusions and histology.

Material input

Living athymic mice grafted with Day 25-35 human iPSC-derived neural progenitor cells.

Experimental Outline

1d 0h 16m

1 Attach a needle into the peristaltic pump tubing and prime the tubing by filling with Room temperature 1x PBS.

2 Lethally overdose mice with sodium pentobarbitone (100 mg/kg) via intraperitoneal injection.

Note

Anaesthetic depth is confirmed by the absence of withdrawal reflex, lack of response to both toe and tail pinch and a low respiratory rate.

3 Working in a fume hood, make a lateral incision just beneath the rib cage, immediately inferior to the xyphoid process.

4 Cut through the diaphragm to expose the thoracic cavity.

5 Cut through the rib cage on the lateral edge, and then reflect the sternum above the head and hold in place with a pair of forceps.

6 Complete occlude the descending aorta using a clamp lacking teeth.

Note

These may prevent full occlusion of the vessel. Gently move the lungs with a micro spatula to uncover the descending aorta.

7 Insert the gavage needle attached to the pump tubing into the apex of the heart, advancing 2 mm - 3 mm into the tissue.

7.1 Clamp the heart and enveloped needle with forceps.

Note

Ensure the tip of the needle is not occluded by clamped forceps.

7.2 Cut the right atrium and turn on the perfusion pump at a rate of approximately 5mL/minute.

8 Perfuse the tissues for 00:05:00 with Room temperature 1x PBS. 5m

9 Crimp the tubing to prevent air bubbles entering the line and then switch the source solution for the perfusion pump from Room temperature 1x PBS to ice cold 4% PFA in 1x PBS.

10 Re-start the perfusion pump at 5mL/minute and perfuse the tissues for a further 00:08:00 . 8m

Note

Fixation tremors should be observed after 00:01:00 - 00:02:00 .

11 Collect and store PFA run-off for disposal.

12 Excise the brain and place immediately in a 50 mL sample container filled with ice-cold 4% PFA in 1x PBS. Place sample container on vertical rocker at 4 °C for up to 24:00:00 . 1d

13 Discard PFA solution into appropriate waste disposal stream.