Intracardiac perfusion and rat brain fixation for immunohistochemistry

In 1 collection

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ABSTRACT

Protocol for rat brain processing to perform parallel histological analysis in one hemisphere and high dimensional flow cytometry in the other.

MATERIALS

Materials:

- peristaltic pump
- surgical instruments
- cryostat

Reagents:

- Phosphate buffer solution (PB, 0.1 M, pH 7.4)
- 4% paraformaldehyde (PFA) in PB (0.1 M, pH 7.4)
- 30% sucrose + 10% glycerol solution in PB (0.1 M, pH 7.4)
- OCT
- 2-methylbutane

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1. Animal sacrifice

5m

1 Before starting, fill the tube of a peristaltic pump with ice-cold 1X PB solution

2 Deeply anesthetize the animal. Check for no reflexes and slower breathing

3 Cut the animal's thorax and perfuse the animal through the left ventricle with ice-cold 1X PB solution, after small cut of the right atrium to allow fluid flux 5m

4 Remove brains from the skull and divide the two hemispheres into two halves along the medial sagittal line (one hemisphere is freshly dissected and processed for high dimensional flow cytometry, and the other one is processed for histological analysis as follow)

2. Postfixation

5 For histological analyses, postfix one brain half in 40 ml formaldehyde solution 4% in PB buffered for 72h, at 4°C (replace every 24 hours with fresh fixative solution)

3. Brain processing for cryostat sectioning

6 Rinse three times in PB and immerse in 30% sucrose + 10% glycerol solution at 4 °C until sinking for cryoprotection

7 Include brains with OCT

7.1 Freeze in 2-methylbutane, cooled into liquid nitrogen, and store at -80 °C

4. Cryostat sectioning

8 Perform sectioning with a cryostat at 30-µm-thickness

8.1 Store sections at 4°C in 0.1 M PB containing 0.02% sodium azide before being further processed for immunohistochemical staining