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Version 2

Rat brain processing for histological analyses (update) V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for rat brain processing in order to perform histological analyses



Rat perfusion

25m

- 1 Deeply anesthetize animals with sodium pentobarbital (50 mg/kg, i.p.)
- 2 Perfuse through the left ventricle with saline [0.9% (wt/vol)] at room temperature (RT)
- 3 Perfuse again with ice-cold formaldehyde solution 4% in PBS buffered for histology

5m

15m

Postfixation

1d

- 4 Remove brains and post-fix them for 24 h in the same fixative

1d

Processing for microtome sectioning

- 5 Wash twice with 0.1 M PBS and process for paraffin embedding following standard procedures (performed by an external facility)

Alternatively, brains could be stored in 0.1 M PBS at 4 °C (not for over a month without changing the PBS) prior paraffin processing

Processing for cryostat sectioning

2d

- 6 Cryoprotect for 24-48 h (until they sink) in 30% sucrose at 4 °C
- 7 Exchange sucrose for 0.1 M PBS
- 8 Immerse brains in cold (-30°C) 2-methylbutane for 30 s and store at -80 °C
- 9 Include in OCT

2d

30s



Sectioning

- 10 Perform sectioning with a sliding microtome at 5- μ m-thickness for paraffin samples or in a cryostat at 20- or 30- μ m-thickness for frozen samples.