Version 2

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

miquel.vila[1]

[1]Vall d'Hebron Research Institute

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ABSTRACT

Protocol for rat brain processing in order to perform histological analyses

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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) 5m

3 Perfuse again with ice-cold formaldehyde solution 4% in PBS buffered for histology 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 2d

7 Exchange sucrose for 0.1 M PBS

8 Immerse brains in cold (-30ºC) 2-methylbutane for 30 s and store at -80 °C 30s

9 Include in OCT

Sectioning

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

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