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Version 1

Rat brain processing for histological analyses V.1

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miquel.vila¹

¹Vall d'Hebron Research Institute



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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

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

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) or store brains in 0.1 M PBS at 4 °C, alternatively (not for over a month without changing the PBS)

Processing for cryostat sectioning

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

Sectioning



- 7 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.