Rat brain processing for histological analyses V.1

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ABSTRACT

Protocol for rat brain processing in order to perform histological analyses

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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.

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