Sep 13, 2022 Version 2

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

In 2 collections

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvwj61wlmk/v2

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DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwj61wlmk/v2

Protocol Citation: Marta Gonzalez-Sepulveda, Joan Compte, Miquel Vila 2022. Rat brain processing for histological analyses (update). **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwj61wlmk/v2Version created by **Marta Gonzalez-Sepulveda**

Protocol status: Working
We use this protocol and it's working

Created: September 13, 2022

Last Modified: November 10, 2023

Protocol Integer ID: 69922

Keywords: rat brain processing for histological analysis, rat brain processing, protocol for rat brain processing, rat brain processing in order, histological analysis, brain

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) 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 (-30°C) 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.