Feb 16, 2025

Coronal cryosectioning of mouse brains

Forked from Coronal cryosectioning of mouse brains

DOI
https://dx.doi.org/10.17504/protocols.io.x54v9rkzpv3e/v1

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Protocol Citation: Yujie Fan, Chelsie Steele 2025. Coronal cryosectioning of mouse brains. **protocols.io https://dx.doi.org/10.17504/protocols.io.x54v9rkzpv3e/v1**

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

Created: January 07, 2025

Last Modified: February 16, 2025

Protocol Integer ID: 117846

Keywords: coronal cryosectioning of mouse brains cryostat, mouse brains cryostat, coronal cryosectioning, mouse brain

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-020495

Disclaimer

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

Cryostat coronal cryosectioning of mouse brain tissue

Materials

Leica Biosystems, CM1950cryostat (Leica Biosystems, CM1950)

Troubleshooting

Before start

PFA-perfused, 30% sucrose-cryoprotected, and flash-frozen mouse brains are stored at -80C until cryosectioning

Procedure

25m

1 Retrieve frozen mouse brain stored at -80 °C .

2 Place brain in cryostat. Set chamber temperature to -16 °C to -20 °C . Set brain holder temperature to -20 °C . Let brain equilibrate to temperature for at least 00:20:00 before sectioning. 20m

3 Insert chuck and blade into cryostat.

4 Apply a drop of OCT on the chuck, and affix brain vertically (olfactory bulbs up).

5 Apply another layer of OCT at the base of the brain, turning the brain-holder with the other hand. Cover the cerebellum and the junction between the cerebellum and cortex with OCT. Allow OCT to freeze solid (~ 00:05:00). 5m

6 Cut 100 micron sections. Adjust angle of medio-lateral and ventro-dorsal axis so that sections are free of cutting artifacts, symmetrical, and well-aligned to reference atlas.

6.1 Use of anti-roll plate is optional and can help reduce rolling of sections, but must be carefully adjusted to avoid cutting artifacts.

7 Collect free-floating sections in 6-well plate.

7.1 Label your plate lid and base with brain/experiment details using lab marker and/or lab tape.

7.2 Pre-fill wells with 3 mL 1X PBS containing 0.01% sodium azide (100mg/L).

7.3 Collect multiple sections per well. Typically the plate is labeled with different brain regions to differentiate each of the wells

8 Use parafilm to seal the lid to the plate (air-tight) and refrigerate at 4 °C until further processing.