Cryoprotection of Mouse Brain

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ABSTRACT

This protocol allows for accurate cryoprotection of mouse brain, post-perfusions, to be used for histology. The methods utilize another PFA fixation step followed by sucrose incubation.

MATERIALS

- Cold Paraformaldehyde (PFA) solution 4% in PBS
- 30% sucrose in phosphate buffered saline (PBS). To make it, mix 30g of sucrose with PBS solution to a total volume of 100ml.
- Dry ice
- Foil
- 15 ml conical tube

MANUSCRIPT CITATION:
Williams GP, Schonhoff AM, Jurkuvenaite A, Gallups NJ, Standaert DG, Harms AS. CD4 T cells mediate brain inflammation and neurodegeneration in a mouse model of Parkinson's disease. Brain. 2021 Aug 17;144(7):2047-2059. doi: 10.1093/brain/awab103. PMID: 33704423; PMCID: PMC8370411.

Schonhoff, A.M., Figge, D.A., Williams, G.P. *et al.* Border-associated macrophages mediate the neuroinflammatory response in an alpha-synuclein model of Parkinson disease. *Nat Commun* **14**, 3754 (2023). https://doi.org/10.1038/s41467-023-39060-w

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

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PROCEDURE

1 Transfer the brain into 5-10ml of 4% PFA solution in PBS for 2 hours at room temperature in a 15 ml conical tube.

2 Transfer the brain into 30% sucrose solution in PBS, wait until it sinks to the bottom for 48-72 hours at 4°C.

3 Freeze brain on dry ice and store at minus -80 °C till sectioning.

4 To freeze the brain cut foil into pieces that will be labeled accordingly. Lay the foil on the top of the dry ice. Put the brain on the foil. Let the brain to freeze. It should change its color to white, and become hard. Wrap the foils edges. Put samples in the box, label the box with your project, and put it to minus -80 °C.