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Cryoprotection of mouse brain tissue

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Protocol status: Working

We use this protocol and it's working

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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

Fixed brains are submerged in 30% sucrose to cryoprotect prior to freezing and cutting cryosections.

Safety warnings

- ! Dispose of fixative-contaminated sucrose and isopentane as hazardous waste or according to your institutional guidelines

Before start

Following perfusion and post-fixation in 4% ice-cold PFA (for 2 hrs, overnight, or as appropriate for intended use):



Procedure

2d 0h 0m 15s

- 1 Make 30% sucrose solution by dissolving  30 g of sucrose (BP220-1, Fisher) in  100 mL of 1X PBS (BP3991, Fisher)  7.4
- 2 Fill tube (20 mL glass scintillation vials or 15 mL conical centrifuge tubes are classically used) with ~  10 mL of sucrose and transfer fixed brain tissue into tube, gently mix by inverting tube several times.
- 3 Incubate at  4 °C until brain sinks (typically ~  48:00:00). 2d
- 4 Prepare superchilled isopentane (2-methylbutane, M1246, Spectrum) by pouring ~  30 mL into 50 ml bottle or beaker. Carefully position beaker on dry ice in a stable manner. Wait until isopentane temperature reaches  -30 °C before proceeding to flash freezing.
- 5 Rinse brain with chilled 1X PBS.
- 6 Dry brain on paper towel by rolling it and gently pressing against paper towel.
- 7 Use long-handled forceps to transfer brain to superchilled isopentane. Let brain freeze for ~  00:00:15 until completely white and bubbling stops. 15s
- 8 Transfer to storage-tube that has been pre-labeled and superchilled on dry ice.
- 9 Transfer to  -80 °C freezer for long-term storage.