Jul 15, 2024

Cryoprotection of mouse brain tissue

DOI
dx.doi.org/10.17504/protocols.io.261ge5x9jg47/v1

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Protocol Citation: Lauren C. Faget, Thomas Hnasko 2024. Cryoprotection of mouse brain tissue. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.261ge5x9jg47/v1**

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

Created: July 15, 2024

Last Modified: July 15, 2024

Protocol Integer ID: 103430

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 intuitional 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) pH 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.