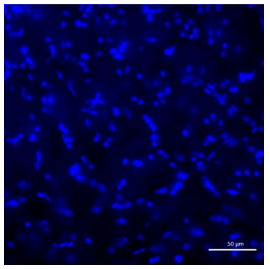
Dec 02, 2024

Postfixation of Snap Frozen Brains



DOI
https://dx.doi.org/10.17504/protocols.io.e6nvw1xx7lmk/v1

Chengjia Qian[1], Olivier George[1], Sonja Plasil[1]

[1]Department of Psychiatry, UCSD School of Medicine

George Lab @ UCSD
Tech. support email: olgeorge@ucsd.edu

Sonja Plasil
Department of Psychiatry, UCSD School of Medicine

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvw1xx7lmk/v1

Protocol Citation: Chengjia Qian, Olivier George, Sonja Plasil 2024. Postfixation of Snap Frozen Brains. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvw1xx7lmk/v1

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

Created: October 22, 2024

Last Modified: December 02, 2024

Protocol Integer ID: 110586

Keywords: Post-fixing, Snap-frozen, Whole brain, PFA, postfixation of snap frozen brain, snap frozen brain, frozen whole brains with pfa, frozen whole brain, downstream cryostat, biological replicate, postfixation, regions of the brain, brain, pfa

Funders Acknowledgements:
- W.M. Keck Foundation Research Program Phase II

Disclaimer

This protocol is only tested in rats, but we have added guideline for mice too for reference.

Abstract

This protocol is used for post-fixing snap-frozen whole brains with PFA, which can be used for downstream cryostat and IHC experiments. It was tested in 1 biological replicate (1 rat) and 3 technical replicates (3 regions of the brain: anterior, medial, posterior).

Attachments

PDF

Images for post-fixi...

4.8MB

Guidelines

This protocol is for rat/mouse that died before being able to perform perfusion. The purpose is to have post-fixed brains comparable to perfused brains when doing IHC experiments and IF imaging.

Materials

4% PFA
30% Sucrose
Sodium Azide
-80C Freezer
-20C Freezer
4C Fridge

Safety warnings

PFA and sodium azide are toxic

Ethics statement

Approval was obtained from Institutional Animal Care and Use Committee (IACUC).

Before start

Make sure you have access to -80C and -20C freezers and 4C fridge
Prepare: (see recipe and steps in protocol)
- 4% PFA in PBS
- 30% sucrose in PBS with 0.1% sodium azide

Protocol

1 Prepare 4% PFA in PBS
(Do all steps in the fume hood. Wear lab coat, mask, and face shield.)

1.1 Set up fume hood. Place hot plate-stirrer in the fume hood. Place 1 L beaker on the hot plate and add a stir bar and thermometer.

1.2 Add ~ 800 mL 1X PBS and 40 g PFA to the beaker and begin heating to 60 °C and stirring to dissolve. DO NOT BOIL.

1.3 If PFA solution looks cloudy, add [M] 1 Mass Percent NaOH dropwise until solution becomes clear.

1.4 Once the solution is clear and PFA is largely dissolved, filter the solution to remove all excess particles (filter paper and conical) and bring the solution to 1L with 1X PBS in a 1L bottle. Place the stir bar inside too.

1.5 Set up and calibrate pH meter. Keep the solution stirring as pH is adjusted. Bring the pH to exactly 7.4 with HCl and NaOH (dropwise).

2 Prepare 30% sucrose in PBS with 0.1% sodium azide

2.1 Dissolve 30 grams of sucrose in PBS (phosphate-buffered saline) to make a final volume of 100 mL. Scale up according to your needs.

2.2 Add 0.1 grams of sodium azide per 100mL of the 30% sucrose in PBS solution.

2.3 Mix thoroughly until everything is completely dissolved.

3 Harvest brain, freeze at -37 °C to -40 °C in 2-methylbutane for 00:00:10 , and store at -80 °C until use.

10s

4 Transfer the snap frozen brain from the -80 °C freezer to the -20 °C freezer. Allow to the brain to change temperature from -80 °C to -20 °C for 24:00:00 . 1d

5 After a minimum of 24 hours, transfer the brain into 4% PFA pH 7.4 at 4 °C . Allow the brain to postfix in 4% PFA pH 7.4 for 72:00:00 at 4 °C . PFA volume should be at least 20x the volume of the tissue (i.e. use a 15 ml conical filled with 4% PFA for a mouse brain and 50ml conical for a rat brain). 3d

6 Switch the brain from 4% PFA at 4 °C to 30% sucrose with 0.1% NaN_3 at 4 °C until the brain sinks (48:00:00). 2d

7 The brain can be stored at 4 °C for up to 1 year. Prepare the brain as normal for your respective sectioning technique.