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# Preparation and cryosectioning of fixed mouse brains

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ABSTRACT

This protocol describes how to prepare thin, fixed mouse brain tissue sections from whole fixed mouse brains in preparation for immunohistochemistry or histology. This process includes immersion fixation and cryoprotection of mouse brains, followed by flash freezing of whole brains and cryosectioning into thin tissue sections using a freezing microtome.

ATTACHMENTS

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MATERIALS TEXT

**Equipment:**

- Small metal ramekin
- Tweezers
- Vertical rocker
- Freezing microtome

**Consumables:**

- **5 mL** , **50 mL** sample containers
- Dry ice
- Foil
- Paint brushes
- Microtome blades (e.g. Epredia MX35 Ultra Low Profile Microtome Blades)

**Key reagents:**

- Isopentane
- Sodium azide
- Sucrose
- Optimal Cutting Temperature (OCT) compound

**Solutions:**

- 10x PBS

1. **77.3 g** of NaH2PO4.H2O ( [M] **0.28 Molarity (M)** ), **203.7 g** of Na2HPO4 ( [M] **0.72 Molarity (M)** ), **177.4 g** of NaCl ( [M] **1.5 Molarity (M)** ) in **2 L** dH2O, pH 6.9

- 1x PBS, pH 7.4

1. **100 mL** of 10x PBS in **900 mL** dH2O, no pH adjustment required

- 30% sucrose in 1x PBS

1. **300 g** sucrose up to **1 L** with 1x PBS

- Anti-freeze solution

1. **300 mL** (30%) glycerol, **300 mL** (30%) ethylene glycol up to **1 L** with 1x PBS

**Material input (animal, cell, tissue, fraction details)**

Whole mouse brains fixed by transcardial perfusion with 4% paraformaldehyde, stored overnight following perfusion in 50mL containers containing 4% paraformaldehyde.

## Cryoprotection and snap freezing

1 Remove fixed brain from 50 mL sample container and discard 4% paraformaldehyde solution into appropriate waste disposal stream.



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2 Place brain back into the **50 mL** sample container and fill with 30% sucrose in 1x PBS containing 0.01% sodium azide.

3 Place container on vertical rocker at **4 °C** for **48:00:00** or until brain no longer floats in sucrose solution. 2d

4 Half fill small metal ramekin (~ **100 mL** capacity) with isopentane (2-methyl-butane) and place on dry ice, allowing it to cool to **-45 °C** .

5 Use plastic tweezers to remove brain from sucrose and immerse in isopentane solution for **00:00:15** - **00:00:20** . 35s

- Monitor solution temperature to ensure it does not drop below **-60 °C** , as temperatures lower than this can cause tissue to crack and fragment.
- Use tweezers lacking teeth, as these will damage/mark the cortex.

6 Remove frozen mouse brains and either'

- Section immediately, or
- Wrap in foil, then wrap in tissue paper, then place in labelled sample tube **-80 °C** for storage.

## Serial cryosectioning of grafted mouse brains for thin tissue histology

7 

If brain tissues are stored at **-80 °C** , move them to **-20 °C** **Overnight** prior to sectioning.

8 Label 12 well microtiter plates with appropriate identifiers (brain tissue IDs, series #s, etc.) and fill each well ⅔ full with anti-freeze solution.

9 Cool freezing microtome sample holder with dry ice.

10 Squeeze OCT onto microtome specimen head until the diameter is approximately the diameter of a **1.5 cm** piece.



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11 Allow OCT to freeze (turns opaque from translucent) then shave layers off by cutting with microtome blade until you have created a flat base, the thickness of which should be a little more than **4 mm** - **5 mm** .

12 Mount caudal end (cerebellum/brainstem) of frozen brain onto flat OCT base using additional OCT compound and non-serrated tweezers.

You may need to gently hold the brain in place until the OCT has frozen.

13 Crush dry ice and surround the OCT embedded brain, pressing gently to compact the dry ice and leaving only the most rostral aspect exposed to begin cutting (olfactory bulb/pre-frontal cortex).

14 Section **30 µm** tissue sections.

- Ensure microtome blade and clamp are slightly wet, this helps to avoid tissue crumpling or sticking to blade.
- Sections must be transferred quickly into wells containing anti-freeze solution using a paint brush.
- If tissue becomes too soft or loses its visual homogeneity due to thawing (sections will start to shred) pause sectioning, top up crushed dry ice around brain sample holder, wait to re-freeze and continue.
- To collect 12 section series', transfer sections sequentially into 12 labelled vials - section 1 is placed into vial 1, section 2 into vial 2, and so on until the last vial is reached, at which point the process begins again and the next section is placed into vial 1 again.

15 Once all sections have been cut for a given brain tissue, store sections in anti-freeze solution at **-20 °C** .



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