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Preparation of Free Floating Coronal Mouse Brain Sections

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details the preparation of free floating coronal mouse brain sections.

Attachments



[prwdbh9k7.pdf](#)

140KB

Materials

Materials:

Cryoprotectant solution

	A	B
	Phosphate buffer	0.1 M
	Sucrose	30%
	Ethylene Glycol	30%

Troubleshooting



Collect Mouse Brain Tissue




2d

- 1 Deeply anesthetize mice via intraperitoneal injection of 2X Avertin solution.
- 2 Perform transcardial perfusion using chilled saline solution
 - 2.1 Keep 0.9% NaCl On ice .
 - 2.2 Use approximately 60 mL saline solution per mouse.
- 3 Switch from saline solution to chilled PFA.
 - 3.1 4% paraformaldehyde in 0.1 Molarity (M) phosphate buffer (PB) 7.4 .
 - 3.2 Use approximately 60 mL PFA per mouse.
- 4 Remove brain immediately after PFA perfusion.
- 5 Incubate brain in PFA for 24:00:00 at 4 °C .

1d
- 6 Transfer brains to 30% Sucrose / 0.1 Molarity (M) Phosphate Buffer (PB) – keep at 4 °C for ≥ 24:00:00 .

1d
- 7 Tissue should be completely saturated with sucrose before sectioning.
 - 7.1 Brains will sink to the bottom of the vial when saturated.

Section Tissue


- 8 Use a Leica SM2010R Microtome.
- 8.1 **Blade:** Leica 16cm, knife angle set at 0 degree.
- 8.2 **Cut thickness:** $\pm 35 \mu\text{m}$.
- 9 Adjust the microtome platform so that it is level with the blade and lock it into place.
- 10 Chill the microtome platform by covering it with crushed dry ice.
- 11 Apply 5-10 drops of 30% Sucrose / **1M 0.1 Molarity (M)** Phosphate Buffer (PB) solution to the chilled microtome platform and wait for it to solidify. 
- 12 Use the microtome to gently shave the solidified sucrose to make a flat surface.
- 13 Use a razor blade to remove any spinal cord from the brain making a flat surface perpendicular to the rostral / caudal axis.
- 14 Apply 2-3 drops of sucrose to the existing sucrose platform and quickly position the brain with the olfactory bulbs pointing upwards. 
- 14.1 Apply 2-3 more drops of sucrose to the top of the brain to securely freeze it to the microtome platform. 
- 15 Gently cover the brain in crushed dry ice to freeze the tissue.
- 16 Adjust the microtome platform so that the rostral / caudal axis is perpendicular to the blade and the dorsal / ventral axis is level with the blade.



- 17 Collect sections in a 24-well plate prefilled with cryoprotectant solution ([M] 0.1 Molarity (M) PB +30% Sucrose + 30% Ethylene Glycol).

Cryoprotectant solution

	A	B
	Phosphate Buffer	0.1 M
	Sucrose	30%
	Ethylene Glycol	30%

- 18 Seal plate with parafilm and store tissue at  -20 °C .