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Preparation of Free Floating Coronal Mouse Brain Sections

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We use this protocol and it's working

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Disclaimer

Abstract

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

Attachments

PDF

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 [M] 0.1 Molarity (M) phosphate buffer (PB) pH 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 / [M] 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:** 35 µm .

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 / [M] 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 .