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

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Preparation of Free Floating Coronal Mouse Brain Sections

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ABSTRACT

Protocol to prepare free floating mouse brain sections for immunostaining or *in situ* hybridization.

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Collect Mouse Brain Tissue

1 Deeply anesthetize mice via intraperitoneal injection of 2X Avertin solution

2 Perform transcardial perfusion using chilled saline solution

2.1 0.9% NaCl kept on ice

2.2 Use approximately 60mL saline solution per mouse

3 Switch from saline solution to chilled PFA

3.1 4% paraformaldehyde in 0.1M phosphate buffer (PB) pH 7.4

3.2 Use approximately 60mL PFA per mouse

4 Remove brain immediately after PFA perfusion

5 Incubate brain in PFA for 24 hours at 4°C

6 Transfer brains to 30% Sucrose / 0.1M Phosphate Buffer (PB) – keep at 4°C for ≥ 24 hours

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: 35µm

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 / 0.1M 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 (0.1M PB +30% Sucrose + 30% Ethylene Glycol)

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

Recipes

19 0.2M Phosphate Buffer (PB)

	A	B
	1L	H2O
	22.72 g	Na2HPO4
	5.52 g	NaH2PO4

20 4% PFA

	A	B
	500mL	8% paraformaldehyde
	500mL	0.2M PB

21 Saline

	A	B
	1L	H2O
	9 g	NaCl

22 30% Sucrose

	300g	Sucrose
	Fill to 1L	0.1M PB

1. Stir and heat to 60°C until dissolved

23 Cryoprotectant Solution

	A	B
	300g	Sucrose
	300mL	Ethylene glycol
	Fill to 1L	0.1M PB

Completely dissolve sucrose in 0.1M PB before adding ethylene glycol. After adding ethylene glycol, fill to 1L with 0.1M PB.