

Brain Slice Preparation

Procedure:

1. Turn on the water bath (35°C).
2. Make 500 ml cutting C solution (prepare fresh daily, see below).
3. Prepare two slice incubation chambers.
4. Fill the incubation chambers and the slice chamber on the vibratome with cutting solution C. Place the incubation chambers in the water bath. Turn on oxygen/CO₂ (see below).
5. Turn on the vibratome (VB 900) to cool down the cutting solution in the slice chamber.
6. Freeze ~250 ml of cutting C solution at -80°C for about 25 minutes.
7. Freeze dissection instruments.
8. Remove cutting C solution from freezer and place on ice.
9. Prepare two boxes of ice, and cool the empty dissection chamber in one box. The other box will be used to cool the tubing of the perfusion pump as well as the tray on which the mouse will be dissected.
10. Anesthetize the mouse using 0.5 ml of 3.8% chloral hydrate.
11. Cool the anesthetized mouse at -20°C for 5 minutes.
12. Retrieve the frozen dissection instruments, and prepare for perfusion and dissection (fill dissection chamber with ice-cold cutting C solution and turn on pump to circulate the solution).
13. Intracardially perfuse the mouse with about 140 ml ice-cold cutting C solution; Rapidly decapitate the mouse, dissect out the whole brain, and place in the cold dissection chamber.
14. Separate the hemibrains using a razor blade.
15. Glue the left hemibrain on the cutting disk, and fix the right hemi-brain in 4% PFA.
16. Slice the hemibrain into 250 µm slices with the vibratome, then remove the frontal portions of the slices using a scalpel and syringe needle.
17. Transfer hippocampal slices to the incubation chamber (arrange in order of brain level) containing bubbling cutting C solution and incubate the slices for 45 minutes at 35°C.
18. Transfer slices into normal external solution and store at room temperature until ready to record.

Cutting solutions:

<u>Cutting A:</u>	<u>for 2L(10x) store at 4°C</u>
NaCl (MW 58.44)	46.7520 g
KCl (MW 74.56)	5.9648 g
NaH ₂ PO ₄ (MW 137.99)	3.4498 g
NaHCO ₃ (MW 84.01)	42.0050 g

<u>Cutting B:</u>	<u>for 500ml (100x) store at 4°C</u>
MgCl ₂ (MW 203.30)	71.1550 g
CaCl ₂ (MW 147.02)	3.6755 g

<u>Cutting C:</u>	<u>for 500 ml (prepare fresh daily)</u>
Cutting A (10x)	50 ml
DDW	400 ml
Sucrose (342.30MW)	24.56 g
Dextrose (180.16MW)	0.9008 g
Ascobate (176.12MW)	0.0881 g
Na Pyrurate (110.00(MW)	0.1650 g
Myo inositol (180.20MW)	0.2703 g

Stir and adjust pH to < 7.5 by bubbling 95% O₂ and 5% CO₂
Add 10 ml Cutting B solution
Add DDW to 500 ml
Measure and adjust osmolarity to 310 mOsm