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Acute Brain Slices

In 1 collection

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ABSTRACT

This protocol describes the steps for preparing acute brain slices.

BEFORE START INSTRUCTIONS

Prepare stock solutions: 10X Stock solutions can be made ahead of time and are shelf stable at Room temperature for 1 month; 1X solutions must be made fresh

10X ACSF

10X Cutting Solution

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Acute Brain Slices

1 Set up vibratome and cutting station:

1.1 Make 1X ACSF:

- Prepare 1 L per mouse.
- 900 mL MilliQH2O + 100 mL stock 10X ACSF + 1.8 g NaHCO3 + 2 g glucose
- Keep in water bath 32 °C and bubble continuously with 95% O_2 / 5% CO_2.
- Add ~30 mL to a slice holding vial, keep at 32 °C and bubble continuously with 95% O_2 / 5% CO_2.

1.2 Make 1X cutting solution:

- Prepare 250 mL per mouse
- 225 mL MilliQH2O + 25 mL stock 10X cutting solution + 0.5 mg NaHCO3 + 0.125 g glucose + 4.3 g sucrose
- Chill on ice and bubble with 95% O_2 / 5% CO_2.

1.3 Set up vibratome

- Carefully clean blade with ethanol and water
- Using a hex wrench, insert blade in blade holder assembly
- Run VibroCheck calibration
- Place buffer tray in ice tray and fill ice tray with ice; load on to vibratome
- Superglue a rectangular piece (~2 cm x 1 cm x 1 cm) of 3% agar onto the specimen plate

1.4 Set up perfusion and cutting tools

2 Perfuse animal with cold cutting solution

3 Dissect brain and glue it to specimen plate.

4 Insert plate in buffer tray and fill tray with 1X cutting solution.

5 Lower blade into solution, set front and back limits and settings.

- Slice at 0.8 mm/s
- 240 µm slices

6 Collect relevant slices as they come off the blade, and carefully transfer to warmed slice vial.

7 Recover slices at 32 °C and bubbled for at least 45 minutes.

Solution preparation

8 10X Cutting solution stock

	A	B	C	D	E
	Compound:	CAS No.	FW:	conc. (mM)	10X Stock (g/2L)
	NaCl	7647-14-5	58.44	75	87.66
	KCl	7447-40-7	74.55	2.5	3.72
	MgCl2*6H2O	7791-18-6	203.3	6	24.4
	CaCl2*2H2O	10035-04-8	147.01	0.1	0.3
	NaH2PO4*H2O	10049-21-5	137.99	1.2	3.32

9 10X ACSF stock

	A	B	C	D	E
	Compound:	CAS No.	FW:	conc. (mM)	10X Stock (g/4L)
	NaCl	7647-14-5	58.44	126	294.52
	KCl	7447-40-7	74.55	2.5	7.44
	MgCl2*6H2O	7791-18-6	203.3	1.2	9.75
	NaH2PO4*H2O	10049-21-5	137.99	1.2	6.64
	CaCl2*2H2O	10035-04-8	147.01	2.5	14.7