

Sectioning Solution (add Calcium and Magnesium last)

Chemical	Molecular Weight	mM	g/500 mL 10x	
Choline Chloride	139.63	110	76.397	
KCl	74.55	3.5	1.304625	
CaCl ₂ 2H ₂ O	147	0.5	0.3675	
NaH ₂ PO ₄ H ₂ O	138	1.3	0.897	
MgCl ₂ 6H ₂ O	203.3	7	7.1155	
			g/500ml 1x	To add day of
NaHCO ₃	84.01	25	0.42	
D-glucose	180.16	20	0.72	
Na-Ascorbate	198.11	11.6	11.4	
Na-Pyruvate	110.04	3.1	1.706	

Recording Solution (ACSF) (add Calcium and Magnesium last)

Chemical	Molecular Weight	mM	g/1000 mL 10x	
NaCl	58.44	125	73.05	
KCl	74.55	3.5	2.60925	
CaCl ₂ 2H ₂ O	147	2	2.94	
NaH ₂ PO ₄ H ₂ O	138	1.3	1.794	
MgCl ₂ 6H ₂ O	203.3	1.3	2.6429	
			g/1000ml 1x	To add day of
NaHCO ₃	84.01	25	2.1	
D-glucose	180.16	10	1.8	

Notes:

Taking Slices:

1. 1000 mL Recording (100ml Rec / 9000ml ddH₂O)
 - a. Oxygenate in incubator (32 deg C for mice <1 month, 34 deg C for mice >1 month)
2. 200 mL Sectioning (20ml Sec/ 180 ml ddH₂O)
 - a. Oxygenate in ice (add Sectioning ice if you have)
3. Prepare agarose square from fridge (add to vibratoam insert) and wash blade and put into vibratoam

4. Fill vibratoam with ice
 - a. Position the blade in second position (barely hiding white mark)
5. Perfuse mice quick (within 1 minute) with sectioning, isolate skull and drop sectioning on top
6. Isolate out brain and submerge in bubbled sectioning bath
7. Add superglue to vibratoam insert with glued on agarose
8. Take brain out and cut off hind brain – adding sectioning as much as possible to atop the brain
9. Insert into vibratoam and take 300 μm slices (can take more/less depending on need) at .09mm/s (can go a .01 mm up or down depending on preference)
10. Put slices onto screen in recording solution in incubator for 45 minutes – 1 hour
11. Switch to room temperature for 15 minutes, then record for 4-6 hours

E-Phys recording Setup:

1. Turn on Digidata
2. Turn on Computer
3. Turn on Scope Light
4. Turn on power box
5. Turn on manipulators (electrode and stage)
6. Put in stage cover, reference electrode and IV heater
7. Fill with recording solution and bubble
8. Turn on Cycler

Electrode Mover	
Left/Right	+/-
Forward/Back	+/-
Up/Down	+/-
In/Out	+/-