

Making NMDG stock:

Protective Recovery from Guoping Feng . (5X, minus bicarbonate) .			
Date Made:			
Final Volume: 1000ml		non-toxic	
Ingredient	mM	MW	Grams
KCl (potassium chloride)	2.5	74.56	0.932
NMDG (N-Methyl-D-glucamine)	92	195.21	89.80
Glucose	25	180.2	22.52
HEPES	20	238.3	23.84
Sodium-Ascorbate	5	198.11	4.952
Sodium Pyruvate	3	110.04	1.650
Thiourea	2	76.12	0.7612
NaH ₂ PO ₄ •H ₂ O (sodium phosphate)	1.2	137.99	0.828
12N HCl	92	36.46	38.02 mL
CaCl ₂ •2H ₂ O	0.5	147.02	0.3672
MgSO ₄ •7H ₂ O (magnesium sulfate)	10	246.48	12.32

- I add chemicals in the order listed on the label
- NMDG is very fluffy, so I normally add some water to it in the weigh boat (~25ml, after weighing) so it is easier to pour into the bottle
- I let everything stir for ~30 minutes after adding the sodium phosphate, so everything is dissolved before adjusting pH
- Volume should be ~900ml at this point
- pH starts out above 10, it takes a decent amount of HCl before it starts dropping, but be careful, it moves more quickly once it's below 9
 - Target pH is 7.3-7.4
- Bring volume to 1L after adding the last two chemicals
- I have noticed that the pH will increase again to >8 within a few days
 - This causes the solution to darken and some chemicals to precipitate out
 - Check pH the day after you make the solution and about once a week following that, adjust as necessary

Making NMDG working solution:

- 500ml is enough for slicing and perfusing two young rats
 - May need more for larger animals
- Start with ~300ml water, 100ml 5X NMDG, 50ml 10X bicarbonate
- Target osmolarity is 300 mOsm/L
- Place NMDG solution in -20 prior to using
 - You want it to be "ice cold" for perfusing, some ice crystals forming, but not frozen solid

Slicing:

- Place 150ml of NMDG solution in a 36°C water bath
- Another 200ml goes in a beaker on ice
- Perfuse animals and slice brains with ice cold NMDG
- Use the last 150ml of cold NMDG to hold your slices
- After collecting last slice, pour off cold NMDG from slice holding container and replace with the warm NMDG from the water bath
- Incubate slices in warm water bath for 15 minutes
 - Slices will float after changing solutions, so make sure to nudge them back down to the mesh
- Transfer slices to room temp ACSF and wait another 45 minutes before recording