Miles Lab Solutions

***** Add all chemicals in the order that they are listed and wait until fully dissolved before adding the next. *****

All solutions are made on a weekly basis and stored at 4°C. For optimal results, recording aCSF should be made daily but can be used for up to a week if stored in the fridge.

When possible, make only enough solution to last the week so there is a weekly turnover of solutions. Ie. Do not make 2 L of solution if only go through 500 mL per week.

Sucrose Dissecting/Slicing aCSF			1000 mL	2000 mL
Compound	MW	Conc. (M)	Weight (g)	Weight (g)
NaCl	58.44	0.025	1.461	2.922
Sucrose	342.3	0.188	64.352	128.704
KCl	74.56	0.0019	0.142	0.284
NaH2PO4	137.99	0.0012	0.166	0.332
MgSO4	120.37	0.01	1.204	2.408
CaCl2	147	0.001	0.147	0.294
NaHCO3	84.01	0.026	2.184	4.368
Glucose	180.16	0.025	4.504	9.008
Kynurenic Acid	189.2	0.0015	0.284	0.568

This solution works well for neonatal spinal cord slices age P0-14. Keeping slicing solutions cold (1-4 degrees C) during dissection and slicing and being fast (20-30 minutes from decapitation to last slice) improves tissue viability.

Recovery aCSF			500 mL	1000 mL
Compound	**MW**	**Conc. (M)**	**Weight (g)**	**Weight (g)**
NaCl	58.44	0.119	3.477	6.954
KCl	74.56	0.0019	0.071	0.142
NaH2PO4	137.99	0.0012	0.083	0.166
MgSO4	120.37	0.01	0.602	1.204
CaCl2	147	0.001	0.074	0.148
NaHCO3	84.01	0.026	1.092	2.184
Glucose	180.16	0.02	1.802	3.604
Kynurenic Acid	189.2	0.0015	0.142	0.284
*Dextran 3%	Add on the day			

*** 3% dextran is added to recovery aCSF on the day of the experiment. Ie. 1.5 g dextran in 50 mL of recovery aCSF.**

Tissue is usually left in warm (35 degrees C) carbogenated recovery solution for 30 minutes after completion of slicing.

Recording aCSF			1000 mL	2000 mL
Compound	**MW**	**Conc. (M)**	**Weight (g)**	**Weight (g)**
NaCl	58.44	0.127	7.422	14.844
KCl	74.56	0.003	0.244	0.488
NaH2PO4	137.99	0.00125	0.172	0.344
MgCl2	203.3	0.001	0.2033	0.4066
CaCl2	147	0.002	0.294	0.588
NaHCO3	84.01	0.026	2.184	4.368
Glucose	180.16	0.01	1.802	3.604

Adult Slicing aCSF			1000 mL
Compound	**MW**	**Conc. (mM)**	**Weight (g)**
K-gluconate	234.2	130	30.446
KCl	74.55	15	1.11825
EGTA	380.4	0.05	0.01902
HEPES	238.3	20	4.76
D-Glucose	180.16	25	4.504
Kyneurenic Acid	189.17	3	0.567
Na-Pyruvate	110.04	2	0.220
Myo-inositol	180.16	3	0.540
Na-L-ascorbate	198.11	1	0.198

pH to 7.4 with NaOH; estimated osmolarity: 345 mOsm

This solution works well for neonatal to young adult spinal cord slices with viable motoneurons up to P20. Interneurons can be studied in vitro in older preparations.

Slicing Adult Tissue

Preparation

- **Pre-freeze blocks of K-gluconate slicing solution in ice cube tray**
- **Place dissecting dish and slicing chamber in freezer at start of day.**
- **Prepare recovery chamber with carbogenated warm recording aCSF (35 degrees C) in water bath**
- **Chill 150mL K-Gluconate aCSF on ice with Carbogen. Add 4-6 K-gluconate ice cubes.**
- **Place 150mL of K-Gluconate slicing solution in the freezer for 45 minutes until slushy - but not too slushy. Too many ice crystals increase the risk of inducing mechanical stress on the cord and also changes in osmolarity of solution when frozen.**
- **Use super glue to secure a block of agar (3-4%) to the cutting base**

- **When ready to dissect, remove the slushy slicing solution and bubble with carbogen for 5 minutes.**

Slicing

- **Dissect spinal cord from animal (3-5 minutes) in ice cold carbogenated K-Gluconate aCSF.**
- **Use a capillary to apply a strip of VetBond surgical glue to the forward-facing surface of the agar block.**
- **Place the spinal cord on top of the glue with the ventral side facing out and caudal end facing up.**
- **Immerse in slushy K-gluconate slicing solution.**
- **Make all possible attempts to reduce compression of the spinal cord when slicing.**
- **Slice spinal cord on slowest possible setting on vibratome (10 um/s).**
- **Transfer slices to the recovery chamber with a pasteur pipette.**
- **Allow slices to recover for 30 minutes after the last slice. I usually take 3-4 slices per spinal cord to minimize slicing time.**
- **Remove the recovery chamber from the water bath and allow it to equilibrate to room temperature for 1 hour prior to starting experiments.**

Approaches were learned during a visit to the Laboratory of Marco Beato (University College London) in August of 2019 and can be found in publications of their work.

KMeSO4 Intracellular Solution			50 mL
Compound	**MW**	**Conc. (M)**	**Weight (g)**
KMeSO4	134.2	0.14	0.9394
NaCl	**58.44**	**0.01**	**0.0292**
CaCl2	147	0.001	0.0074
HEPES	238	0.01	0.1190
EGTA	380	**0.001**	0.0190
Mg-ATP	507.2	0.003	0.0761
Sucrose	342.3	0.005	0.0456
		Stock conc. (M) Final conc. (M)	Volume (0.25 mL H2O) add (g)
GTP-Na3 stock	523.2	0.045 0.0004	0.0059

pH KMeSO4 ICS to 7.25 with KOH, ~0.8 mL of 0.5M KOH

Freeze KMeSO4 in 1mL aliquots and GTP Stock into 10 uL aliquots.

On day of experiment, thaw 1 aliquot of each and add 9uL GTP stock to 1 mL aliquot of KMeSO4 intracellular solution.

CsMeSO4 Intracellular Solution			50 mL
Compound	**MW**	**Conc. (M)**	**Weight (g)**
CsmeSO4	228	0.1	1.1400
TEA-Cl	165.7	0.03	0.2486
NaCl	**58.44**	**0.01**	**0.0292**
CaCl2	147	0.001	0.0074
HEPES	238	0.01	0.1190
EGTA	380	**0.001**	0.0190
Mg-ATP	507.2	0.003	0.0761
Sucrose	342.3	0.008	0.0456
		Stock conc. (M) Final Conc. (M)	Volume (0.25 mL H2O) add (g)
GTP-Na3 stock	523.2	0.045 0.0004	0.0059

pH CeMeSO4 ICS to 7.25 with KOH, ~1 mL of 0.5M KOH

Freeze CsMeSO4 in 1mL aliquots and GTP Stock into 10 uL aliquots.

On day of experiment, thaw 1 aliquot of each and add 9uL GTP stock to 1 mL aliquot of KMeSO4 intracellular solution.