## Xu lab protocol and instructions

## INTRACELLULAR SOLUTION FOR BRAIN SLICES Cesium Gluconate solution Xu Lab

A modified recipe by Xu Lab 081709

Take fix free glassware and add:

Substance	Concentration (mM)	MW	Amount/50mL
CsOH monohydrate	130	167.93	1.0915g
D-Gluconic acid (50%)	130	196.2	2.074ml (1.23 g/ml)
EGTA	0.2	380.4	0.0038g
MgCl <sub>2</sub>	2	95.21	0.1 mL
CsCl	6	168.4	0.05052g
HEPES (free acid)	10	238.3	0.1192g

Add substances to 45mL of the DD H<sub>2</sub>O initially.

pH would be good around 8.5. You can stabilize it using CsOH and Gluconic acid.

Then add following Sigma compounds located in the -20 C freezer:

Substance	Concentration (mM)	MW	Amount/50mL
Adenosine 5' Triphosphate Sodium Salt (A7699-1G)	2.5	551.1	0.0689g
Guanosine 5' Triphosphate sodium salt hydrate Minimum 95% (G8877-25MG)	0.5	523.2	0.0131g
Phosphocreatine disodium salt hydrate enzymatic approx. 98% (P7936-1G)	10	255.1	0.12755g

Check Osmolarity. This should be approximately  $\sim$ 300-305. You can stabilize using water or D-gluconate acid. In general,  $\sim$ 95% difference between extracellular and intracellular solutions (recording aCSF is  $\sim$  305 Osm).

Then add enough water to bring the solution amount to the desired amount (50 mL).

After solution has stabilized, take a 1 mL sample, and check pH and Osmolarity again. pH should be  $\sim$ 7.2 – 7.3 and Osmolarity should be 300-305.

Place the solution into 1mL vials and store in -80 Celsius freezer.

Biocytin concentration (% and mM)

1%	(10mg)	= 26mM
0.75%	(7.5mg)	= 20mM
0.5%	(5mg)	= 13mM