Intracellular Solution Protocol 3/14/2019

- Calibrate the microsomometer
  - Turn on
  - Run diagnostic
  - Run calibration
    - Use calibration standard solution
      - 50 x3
      - 850 x3
      - 2000 x3
- Make stocks fresh **EVERYTIME**
  - Best way to measure stocks is to put the entire tube in to the weigher, then add ddH20
- For pH
  - Calibrate the pH before use here every time
  - For **EPSC** and **IPSC** uses **cesium-hydroxide** to increase pH
  - For **K-ICS** use **potassium-hydroxide** to increase pH
  - Use **HCL** to lower pH for **all**
  - **pH must be 7.35**
- Keep all solutions below 50 mL, around 40 mL for pH and osmolality adjustment
- For osmole test you can only lower the osmoles so **CAREFULLY APPROACH AIM (290 mOSM)**
  - To adjust
    - $Current\ Osmole\ (363) \times current\ volume\ (43) = 15,609$
    - $\frac{15,609}{Aim\ Osmoles\ (290)} = 53.82$
    - $53.82 - current\ volume\ (43) = 10.824$
    - Add 10.824 µL **BUT** add less than that as you can only go down with osmoles, once past that's it

EPSC	mM	MW	g/50ml	stock	Check	Check	Check
Cs-methanesulfonate (CsCH3SO3)	125	228	1.425				
CsCl	5	168.36	0.04209	2.5 ml			
HEPES	10	238.31	0.119155	0.5 ml			
phosphocreatine	10	453.4	0.2267				
EGTA	0.2	380.35	0.0038035	0.1 ml			
ATP Na	4	551.14	0.110228				
GTP Na	0.3	523	0.007845	0.75 ml			
QX314	5	343.3	0.085825				
MgCl2.6H2O	4	203.3	0.04066	0.2 ml			
IPSC	mM	MW	g/50ml				
Cs-methanesulfonate (CsCH3SO3)	90	228	1.026				
CsCl	40	168.36	0.33672				
HEPES	10	238.31	0.119155	0.5 ml			
phosphocreatine	10	453.4	0.2267				
EGTA	0.2	380.35	0.0038035	0.1 ml			
ATP Na	4	551.14	0.110228				
GTP Na	0.3	523	0.007845	0.75 ml			
QX314	5	343.3	0.085825				
MgCl2.6H2O	4	203.3	0.04066	0.2 ml			
K ICS	mM	MW	g/50ml				
K-gluconate	125	234.2	1.46375				
KCl	5	74.55	0.0186375	0.25 ml			
HEPES	10	238.31	0.119155	0.5 ml			
phosphocreatine Na2	10	453.4	0.2267				
EGTA	0.2	380.4	0.003804	0.1 ml			
ATP-Na 2	4	551.4	0.11028				
GTP-Na 3	0.3	523.2	0.007848	0.75 ml			
MgCl 2 •6H 2 O	4	203.3	0.04066	0.2 ml			
pH7.35,290mOsm							
Stock (M)		g/10ml	Add in (ml)				
0.1	CsCl	0.17	2.50				
1	HEPES	2.38	0.50				
1	Mgcl2	2.03	0.20				
0.1	EGTA	0.38	0.10				
0.02	GTP-Na	0.10	0.75				
1	KCl	0.7455	0.25				