

Solutions for Electrophysiology

External Solution (for Patch Clamp Recording)

	Conc. (mM)	FW (g/mol)	1000ml (g)	500ml (g)
NaCl	140	58.44	8.182	4.091
KCl	2.8	74.55	0.2087	0.1044
HEPEs	10	238.3	2.383	1.192
MgCl ₂ *6H ₂ O	1	203.3	0.2033	0.1017
			(10 ml 100mM stock)	(5 ml 100mM stock)
CaCl ₂ *2H ₂ O	2	147.0	0.2940	0.1470
			(20 ml 100mM stock)	(10 ml 100mM stock)

PH 7.2~7.4, adjust with NaOH

OSM 290~310

Add D-(+)-Glucose (MW 180.2) 10 mM before use, ~2 mg/ml.

Internal Solution (for Patch Clamp Recording)

	Conc. (mM)	FW (g/mol)	100ml (g)	50ml (g)
NaCl	10	58.44	0.05844	0.02922
KCl	145	74.55	1.081	0.5405
HEPEs	10	238.3	0.2383	0.1192
MgCl ₂ *6H ₂ O	1	203.3	0.02033	0.01017
			(1 ml 100mM stock)	(0.5 ml 100mM stock)

PH 7.2~7.4, adjust with KOH

OSM 290~310* (usually OSM of the internal solution is 10 lower than external solution)

Add EGTA 1 mM before use.

EGTA was prepared as 100mM in stock, so add 10μl to 1ml internal solution before use.

Cs-Glutamate Internal Solution (for Patch Clamp Recording)

	Conc. (mM)	FW (g/mol)	100ml (g)	50ml (g)
NaCl	10	58.44	0.05844	0.02922
GlutamicAcid	145	147.1	2.132	1.066
HEPEs	10	238.3	0.2383	0.1192
MgCl ₂ *6H ₂ O	1	203.3	0.02033	0.01017
			(1 ml 100mM stock)	(0.5 ml 100mM stock)

Titrate with 5M CsOH to PH 7.2~7.4

OSM 290~310

Add ATP 2 mM, GTP 0.5 mM before use.

ATP/GTP was prepared as 200mM/50mM, 5μl per tube in stock, so add 500μl internal solution before use.

High K⁺ Solution (for secretion stimulus)

	Conc. (mM)	FW (g/mol)	100ml (g)	50ml (g)
NaCl	40	58.44	0.2338	0.1169
KCl	100	74.55	0.7455	0.3728
HEPEs	10	238.3	0.2383	0.1192
MgCl ₂ *6H ₂ O	1	203.3	0.02033 (1 ml 100mM stock)	0.01017 (0.5 ml 100mM stock)
CaCl ₂ *2H ₂ O	2	147.0	0.02940 (2 ml 100mM stock)	0.01470 (1 ml 100mM stock)

PH 7.2~7.4, adjust with KOH
OSM 290~310
Add D-(+)-Glucose (MW 180.2) 5 mM before use, ~1 mg/ml.

10 μM Ca²⁺ Buffer Internal Solution (for secretion stimulus)

	Conc. (mM)	FW (g/mol)	1000ml (g)	500ml (g)
NaCl	10	58.44	0.5844	0.2922
MgCl ₂ *6H ₂ O	1	203.3	0.2033 (10 ml 100mM stock)	0.1017 (5 ml 100mM stock)
CaCl ₂ *2H ₂ O	44	147.0	6.468	3.234
HEDTA	60	278.3	16.70	8.349
Glutamic Acid	50	147.1	7.355	3.678

Titrate with CsOH*H₂O to PH 7.2~7.4
OSM 290~310
Add MgATP 4mM before use.
HEDTA K_{diss}=3.32u
*This solution is used to induce the cell to secrete after whole cell patch clamp, the concentration of [Ca²⁺]_{free} is set at around 10 μM, and the buffer capacity is set at larger than 1 K.

Ferricyanide Solution (for Carbon Fiber Electrode Testing)

	Conc. (mM)	FW (g/mol)	40ml (g)	20ml (g)
Ferricyanide	1	329.2	0.0132	0.0066
KCl	500	74.55	1.1491	0.7455

PH 3.0
Ferricyanide and KCl are dissolved in ddH₂O, use HCl to adjust PH

Solutions to adjust PH

	Conc. (M)	FW (g/mol)	50ml (g)
NaOH	1 M	40	2
KOH	1 M	56.11	2.8
HCl	5 M		

Stock Solutions

	Stock Conc.	FW (g/mol)	Prepare	Notes
MgCl ₂ *6H ₂ O	100 mM	203.3	1.017g/50ml	
CaCl ₂ *2H ₂ O	100 mM	147.0	0.735g/50ml	
EGTA	100 mM	380.4	0.3804g/10ml	Used 1mM in pipette solution to chelate Ca.
EDTA				
TEA-Cl	1 M			Used 10mM in bath solution to block K channels
TTX	10 mM			Used 10μM in bath solution to block Na channels
Amphotericin B	50 mg/ml in DMSO	924.1	50 mg/ml in DMSO	Final conc. is 0.5mg/ml, 0.54mM. Add 10μl to 1ml pipette solution before use
5-HT (serotonin)	60 mM	202.7	12.8 mg/1ml	Add 10μl to 1 ml culture medium and incubate overnight as 0.6mM

MgATP Solution in Stock (keep in -20°C, put on ice when use)

Concentration in stock: 400 mM, store in 5 μl/tube

Concentration in use: 4 mM, Dilute 100 times, add 500 μl to the tube before use

MgATP MW: 507.2

20mg MgATP can be dissolved in 98.6 μl ddH₂O, 50 mg in 246.5 μl ddH₂O

ATP/GTP Solution in Stock (keep in -20°C, put on ice when use)

Concentration in stock: 200mM/50mM, store in 5 μl/tube

Concentration in use: 2mM/0.5mM, Dilute 100 times, add 500 μl to the tube before use

MgATP FW: 507.2, NaGTP FW: 523.2

20mg MgATP can be dissolved in 197μl ddH₂O, 5 mg NaGTP in 191μl ddH₂O.

for easy preparation, weigh 10 mg NaGTP and add 40 mg MgATP

Perforated Patch solutionsPipette Internal Solution:

Same as the Cs-Glutamate Internal Solution, no ATP/GTP added. Add 0.5mg/ml amphotericin B before use.

Amphotericin B:(VWR EM-2500)

50 mg/ml in DMSO and stored at 4°C, protected from light.

Fresh perforated patch pipette solution was prepared every day by addition of 5μl stock amphotericin B to 0.5 ml Cs-Glutamate internal solution. The combined solution was sonicated thoroughly, protected from light and kept on ice. Pipettes were tip-dipped in amphotericin-free solution for 2-10 s and back-filled with freshly mixed amphotericin-containing solution. The liquid junction potential between the extracellular Ringer and the perforated patch solution was measured to be 13.7 mV, and all potentials are adjusted accordingly. (Smith and Neher, 1997)

Smith, C., and Neher, E. (1997). Multiple forms of endocytosis in bovine adrenal chromaffin cells. *J Cell Biol* 139, 885-894.