

Materials List for:

Functional Evaluation of Biological Neurotoxins in Networked Cultures of Stem Cell-derived Central Nervous System Neurons

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Materials

Name	Company	Catalog Number	Comments
Table 1. ESC and ESN			
Knockout DMEM	Life Technologies	10829-018	Media: ESC culture medium Formulation and notes: 500 mL
100x MEM NEAA	Life Technologies	11140-050	Media: store at 4 °C for up to 1 month Formulation and notes: 6 mL
200 mM L-Alanyl-L-Glutamine	ATCC	30-2115	6 mL
ES qualified FBS	Applied Stem Cell	ASM-5007	90 mL
100x antibiotics	Sigma-Aldrich	A5955	3 mL
55 mM 2-mercaptoethanol	Life Technologies	21985-023	1.1 mL
10 ⁷ Units/mL LIF	Millipore	ESG1107	60 µL
Knockout DMEM	Life Technologies	10829-018	Media: ESC differentiation medium Formulation and notes: 436.6 mL
100x MEM NEAA	Life Technologies	11140-050	Media: store at 4 °C for up to 1 month Formulation and notes: 5 mL
200 mM L-Alanyl-L-Glutamine	ATCC	30-2115	5 mL
ESC-qualified serum	Applied Stem Cell	ASM-5007	50 mL
100x antibiotics	Sigma-Aldrich	A5955	2.5 mL
55 mM 2-mercaptoethanol	Life Technologies	21985-023	0.9 mL
Dulbecco's PBS	Sigma-Aldrich	D8537	Media: NPC trypsinization medium Formulation and notes: 100 mL
0.5 M EDTA (18.3%)	Sigma-Aldrich	3690	Media: freeze in 5 mL aliquote Formulation and notes: 0.266 mL
Trypsin	Sigma-Aldrich	T8802	50 mg
Polyethyleneimine (PEI)	Sigma-Aldrich	P3143	Media: Surface coating solutions Formulation and notes: 2.5 µg/mL in H ₂ O
Poly-D-lysine (PDL)	Sigma-Aldrich	P7280	Media: use within 1 wk Formulation and notes: 100 µg/mL in H ₂ O
Laminin	Sigma-Aldrich	L2020	5 µg/mL in H ₂ O
DMEM/F12 + GlutaMAX	Life Technologies	10565-018	Media: NPC culture medium Formulation and notes: 492.5 mL
100x N2 vitamins	Life Technologies	17502-048	Media: store at 4 °C for up to 1 month Formulation and notes: 5 mL
100x antibiotics	Sigma-Aldrich	A5955	2.5 mL

Name	Company	Catalog Number	Comments
Neurobasal A	Life Technologies	10888-022	Media: ESN culture medium Formulation and notes: 482.5 mL
50x B27 vitamins	Life Technologies	17504-044	Media: store at 4 °C for up 1 month Formulation and notes: 10 mL
200 mM L-Alanyl-L-Glutamine	ATCC	30-2115	5 mL
100x antibiotics	Sigma-Aldrich	A5955	2.5 mL
5-fluoro-2'-deoxyuridine (5FDU)	Sigma-Aldrich	F0503	Media: 2000x Mitotic inhibitors aliquot and store at -20 °C Formulation and notes: dd 150 mg 5FDU and 350 mg uridine to 10 mL Neurobasal A. Sterile filter, aliquot and freeze. Use 2000x in ESN culture medium.
Uridine	Sigma-Aldrich	U3003	
TrypLE Express Trypsin	Life Technologies	12605-010	Media: Miscellaneous Formulation and notes: store at RT
DMSO	Sigma-Aldrich	D8418	store at RT
soybean trypsin inhibitor (STI)	Sigma-Aldrich	T6414	store at -20 °C
ethanol	Sigma-Aldrich	E7023	store at RT
ascorbic acid	Sigma-Aldrich	A4403	Prepare 100 mM stock by dissolving 100 mg ascorbic acid in 5.7 mL of 50:50 DMSO/ethanol mix.
retinoic acid (RA)	Sigma-Aldrich	R2625	Resuspend 15 mg RA in 9 mL 50:50 DMSO/ethanol. Supplement with 1 mL of 100 mM ascorbic acid stock. Aliquot and stored at -80 °C. Stable for 6 mos.

Table 2. MIST

NaCl	Sigma-Aldrich	71386	Media: Extracellular Recording Buffer Final Concentration (mM): 58.44 MW: 140
KCl	Sigma-Aldrich	60135	Media: (ERB; pH = 7.3, 315 mO) Final Concentration (mM): 74.56 MW: 3.5
NaH ₂ PO ₄	Sigma-Aldrich	S3139	Media: Stable at 4 °C for at least 6 mo. Final Concentration (mM): 119.98 MW: 1.25
CaCl ₂	Sigma-Aldrich	21115	Final Concentration (mM): 110.98 MW: 2
MgCl ₂	Sigma-Aldrich	63069	Final Concentration (mM): 95.21 MW: 1
D-glucose	Sigma-Aldrich	G8644	Final Concentration (mM): 180.16 MW: 10
HEPES	Sigma-Aldrich	H0887	Final Concentration (mM): 238.3 MW: 10
K-gluconate	Sigma-Aldrich	P1847	Media: Intracellular Recording Buffer Final Concentration (mM): 234.5 MW: 140
NaCl	Sigma-Aldrich	71386	Media: (IRB; pH = 7.3, 320 mO) Final Concentration (mM): 58.44 MW: 5

Mg-ATP	Sigma-Aldrich	A9187	Media: Stable at 4 °C for at least 6 mo. Final Concentration (mM): 507.18 MW: 2
Li-GTP	Sigma-Aldrich	G5884	Final Concentration (mM): 523.18 MW: 0.5
CaCl ₂	Sigma-Aldrich	21115	Media: Stable at 4 °C for at least 6 mo. Final Concentration (mM): 110.98 MW: 0.1
MgCl ₂	Sigma-Aldrich	63069	Media: Stable at 4 °C for at least 6 mo. Final Concentration (mM): 95.21 MW: 1
EGTA	Sigma-Aldrich	E3889	380.35
HEPES	Sigma-Aldrich	H0887	Media: Stable at 4 °C for at least 6 mo. Final Concentration (mM): 238.3 MW: 10
bicuculline	Tocris	131	Media: Miscellaneous Final Concentration (mM): 0.01 MW: 417.85
CNQX	Sigma-Aldrich	C239	Final Concentration (mM): 0.01 MW: 276.12
Tetrodotoxin	Sigma-Aldrich	A8001	Final Concentration (mM): 0.005 MW: 645.74
BoNT/A-/G	Metabio	N/A	Media: Final Concentration (mM): TBD MW: 150,000
Tetanus toxin	Sigma-Aldrich	T3194	Final Concentration (mM): TBD MW: 150,000
Sigmacote	Sigma-Aldrich	SL-2	Final Concentration (mM): N/A MW: N/A

Table 3. Equipment and Software

MiniAnalysis (version 6.0.7)	Synaptosoft, Inc.	N/A	Event detection software
Igor Pro (version 6.22A)	WaveMetrics	N/A	Software for visualization of recordings
Patchmaster	Heka	N/A	Data acquisition software
Olympus IX51 inverted microscope	Olympus	N/A	
micromanipulator	Sutter Instrument	MPC-365	
patch-clamp amplifier	Heka	ECB10USB	
micropipette puller	Sutter Instrument	P-1000	
borosilicate capillary tubes	Sutter Instrument	B150-86-10	Corning 7740
18 mm glass coverslips	Fisher	12-545-84	
plasma cleaner	Harrick Plasma	PDC-32G	
cell strainer (40 µm)	Fisher	08-771-1	
Stovall Belly Dancer Shaker	Fisher	15-453-211	
low adhesion dishes	Fisher	05-539-101	Corning 3262
bacterial dishes	VWR	25384-302	100 x 15 mm