

Materials List for:

Conversion of Human Induced Pluripotent Stem Cells (iPSCs) into Functional Spinal and Cranial Motor Neurons Using PiggyBac Vectors

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URL: <https://www.jove.com/video/59321>

DOI: [doi:10.3791/59321](https://doi.org/10.3791/59321)

Materials

Name	Company	Catalog Number	Comments
5-Bapta	Sigma-Aldrich	A4926-1G	chemicals for electrophysiological solutions
Accutase	Sigma-Aldrich	A6964-100ML	Cell dissociation reagent
anti-CHAT	EMD Millipore	AB144P	Anti-Choline Acetyltransferase. Primary antibody used in immunostaining assays. RRID: AB_2079751; Lot number: 2971003
anti-goat Alexa Fluor 488	Thermo Fisher Scientific	A11055	Secondary antibody used for immunofluorescence assays. RRID: AB_2534102; Lot number: 1915848
anti-mouse Alexa Fluor 647	Thermo Fisher Scientific	A31571	Secondary antibody used for immunofluorescence assays. RRID: AB_162542; Lot number: 1757130
anti-Oct4	BD Biosciences	611202	Primary antibody used in immunostaining assays. RRID: AB_398736; Lot number: 5233722
anti-Phox2b	Santa Cruz Biotechnology, Inc.	sc-376997	Primary antibody used in immunostaining assays. Lot number: E0117
anti-rabbit Alexa Fluor 594	Immunological Sciences	IS-20152-1	Secondary antibody used for immunofluorescence assays
anti-TUJ1	Sigma-Aldrich	T2200	Primary antibody used in immunostaining assays. RRID: AB_262133
B27	Miltenyi Biotec	130-093-566	Serum free supplement for neuronal cell maintenance
BamBanker	Nippon Genetics	NGE-BB02	Cell freezing medium, used here for motor neuron progenitors
BDNF	PreproTech	450-02	Brain-Derived Neurotrophic Factor
Blasticidin	Sigma-Aldrich	203350	Nucleoside antibiotic that inhibits protein synthesis in prokaryotes and eukaryotes
BSA	Sigma-Aldrich	A2153	Bovine Serum Albumin. Blocking agent to prevent non-specific binding of antibodies in immunostaining assays

CaCl ₂	Sigma-Aldrich	C3881	chemicals for electrophysiological solutions
Clampex 10 software	Molecular Devices	Clampex 10	Membrane currents recording system
Corning Matrigel hESC-qualified Matrix	Corning	354277	Reconstituted basement membrane preparation from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma. Used for adhesion of iPSC to plastic and glass supports
CRYOSTEM ACF FREEZING MEDIA	Biological Industries	05-710-1E	Freezing medium for human iPSCs
D-Glucose	Sigma-Aldrich	G5146	chemicals for electrophysiological solutions
DAPI powder	Roche	10236276001	4',6-diamidino-2-phenylindole. Fluorescent stain that binds to adenine-thymine rich regions in DNA used for nuclei staining in immunofluorescence assays
DAPT	AdipoGen	AG-CR1-0016-M005	Gamma secretase inhibitor
Dispase	Gibco	17105-041	Reagent for gentle dissociation of human iPSCs
DMEM/F12	Sigma-Aldrich	D6421-500ML	Basal medium for cell culture
Doxycycline	Sigma-Aldrich	D9891-1G	Used to induce expression of transgenes from epB-Bsd-TT-NIL and epB-Bsd-TT-NIP vectors
DS2U	WiCell	UWWC1-DS2U	Commercial human iPSC line
E.Z.N.A Total RNA Kit	Omega bio-tek	R6834-02	Kit for total extraction of RNA from cultured eukaryotic cells
GDNF	PreproTech	450-10	Glial-Derived Neurotrophic Factor
Gibco Episomal hiPSC Line	Thermo Fisher Scientific	A18945	Commercial human iPSC line
Glutamax	Thermo Fisher Scientific	35050038	An alternative to L-glutamine with increased stability. Improves cell health.
Hepes	Sigma-Aldrich	H4034	chemicals for electrophysiological solutions
iScript Reverse Transcription Supermix for RT-qPCR	Bio-Rad	1708841	Kit for gene expression analysis using real-time qPCR
iTaq™ Universal SYBR Green Supermix	Bio-Rad	172-5121	Ready-to-use reaction master mix optimized for dye-based quantitative PCR (qPCR) on any real-time PCR instrument
K-Gluconate	Sigma-Aldrich	G4500	chemicals for electrophysiological solutions
KCl	Sigma-Aldrich	P9333	chemicals for electrophysiological solutions
L-ascorbic acid	LKT Laboratories	A7210	Used in cell culture as an antioxidant
Laminin	Sigma-Aldrich	11243217001	Promotes attachment and growth of neural cells in vitro
Laser scanning confocal microscope	Olympus	iX83 FluoView1200	Confocal microscope for acquisition of immunostaining images
Mg-ATP	Sigma-Aldrich	A9187	chemicals for electrophysiological solutions

MgCl ₂	Sigma-Aldrich	M8266	chemicals for electrophysiological solutions
Mounting Medium	Ibidi	50001	Mounting solution used for confocal microscopy and immunofluorescence assays
Multiclamp patch-clamp amplifier	Molecular Devices	700B	Membrane currents recording system
Na-GTP	Sigma-Aldrich	G8877	chemicals for electrophysiological solutions
NaCl	Sigma-Aldrich	71376	chemicals for electrophysiological solutions
NEAA	Thermo Fisher Scientific	11140035	Non-Essential Amino Acids. Used as a supplement for cell culture medium, to increase cell growth and viability.
Neon 100 µL Kit	Thermo Fisher Scientific	MPK10096	Cell electroporation kit
Neon Transfection System	Thermo Fisher Scientific	MPK5000	Cell electroporation system
Neurobasal Medium	Thermo Fisher Scientific	21103049	Basal medium designed for long-term maintenance and maturation of neuronal cell populations without the need for an astrocyte feeder layer
NutriStem-XF/FF	Biological Industries	05-100-1A	Human iPSC culture medium
Paraformaldehyde	Electron Microscopy Sciences	157-8	Used for cell fixation in immunostaining assays
PBS	Sigma-Aldrich	D8662-500ML	Dulbecco's Phosphate Buffer Saline, with Calcium, with Magnesium
PBS Ca ²⁺ /Mg ²⁺ free	Sigma-Aldrich	D8537-500ML	Dulbecco's Phosphate Buffer Saline, w/o Calcium, w/o Magnesium
Penicillin/Streptomycin	Sigma-Aldrich	P4333-100ML	Penicillin/Streptomycin solution used to prevent cell culture contamination from bacteria.
poly-ornithine	Sigma-Aldrich	P4957	Promotes attachment and growth of neural cells in vitro
SU5402	Sigma-Aldrich	SML0443-5MG	Selective inhibitor of vascular endothelial growth factor receptor 2 (VEGFR-2)
Triton X-100	Sigma-Aldrich	T8787	4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol, t-Octylphenoxy polyethoxyethanol, Polyethylene glycol tert-octylphenyl ether. Used for cell permeabilization in immunostaining assays
Upright microscope	Olympus	BX51VI	Microscope for electrophysiological recording equipped with CoolSnap Myo camera
Y-27632 (ROCK inhibitor)	Enzo Life Sciences	ALX-270-333-M005	Cell-permeable selective inhibitor of Rho-associated, coiled-coil containing protein kinase (ROCK). Increases iPSC survival
µ-Slide 8 Well	Ibidi	80826	Support for high-end microscopic analysis of fixed cells