

Materials List for:

Simple Generation of a High Yield Culture of Induced Neurons from Human Adult Skin Fibroblasts

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Materials

Name	Company	Catalog Number	Comments
Cell Lines			
Adult human dermal fibroblasts			[C2 passage #7] Donor was a 67 year old female. Cells obtained from the Parkinson's Disease Research and Huntington's disease clinics at the John van Geest Centre for Brain Repair (Cambridge, UK).
Reagents for Fibroblast Culture, Transduction and Conversion			
Dulbecco's phosphate-buffered saline (DPBS) [-CaCl ₂ , -MgCl ₂]	Gibco	14190094	
Trypsin-EDTA [0.5%]	Gibco	15400-054	Dilute to 0.05% in DPBS.
Virkon (agent used to neutralize virus)	Viroderm	7511	Dilute to 1% solution with warm water.
Milli-Q Water	Millipore		
Basal medium - Dulbecco's Modified Eagle Medium (DMEM) + GlutaMax	Gibco	61965059	
Penicillin/Streptomycin [10,000 U/mL]	Gibco	15140-122	
Fetal Bovine Serum (FBS)	Gibco	10270-106	
CryoMACS® dimethyl sulfoxide (DMSO) 10	Miltenyi	170-076-303	
Neural differentiation medium - NDiff 227	Takara-Clontech	Y40002	
LM-22A4	Tocris	4607	Dilute 10 mg in 1450 µL DMSO. Stock concentration: 20 mM.
Glial cell line-derived neurotrophic factor (GDNF) [recombinant human]	R&D systems	212-GD-010	Dilute 10 ug in 500 µL 0,1% BSA in DPBS. Stock concentration: 20 µg/mL.
NT3 [recombinant human]	R&D systems	267-N3-025	Dilute 25 µg in 2,5 mL 0,1% BSA in DPBS. Stock concentration: 10 µg/mL.
db-cAMP	Sigma Aldrich	D0627	Dilute 1 g in 40,7 mL Milli-Q water. Filter and make 500 µL aliquots or stock tubes of 10 mL. Stock concentration: 50 mM.
CHIR99021	Axon	1386	Dilute 2 µg in 429,8 µL DMSO. Stock concentration: 10 mM.

SB-431542	Axon	1661	Dilute 5 mg in 595 μ L DMSO. Stock concentration: 20 mM.
Noggin [recombinant human]	Miltenyi	130-103-456	Dilute 100 μ g in 100 μ L of Milli-Q water + 900 μ L 0,1% BSA in DPBS. Stock concentration: 100 μg/mL.
LDN-193189	Axon	1509	Dilute 2 mg in 360 μ L DMSO. Stock concentration: 10 mM.
Valproic acid sodium salt (VPA)	Merck Millipore	676380	Dilute 5 g in Milli-Q water to achieve a stock concentration of 1 M. CAUTION: Avoid ingestion, contact with skin, and breathing dust formation.
Reagents for Coatings			
Gelatin	Sigma Aldrich	G2500	Dilute to 0.1% in Milli-Q water.
Poly-L-ornithine	Sigma Aldrich	P3655	Dissolve in Milli-Q water. Use at 15μg/mL.
Fibronectin	ThermoFisher Scientific	33010-018	2 mL of Milli-Q water + 70 μ L 0,25 M NaOH. Use at 5 μg/mL.
Laminin	ThermoFisher Scientific	23017-015	Store at -80°C. Thaw on ice, keep cool and aliquot 30 μ L. Use at 5 μg/mL.
Reagents for Fluorescence Activated Cell Sorting			
Cell dissociation agent - Accutase (Stem Pro)	ThermoFisher Scientific	A1110501	
Hanks' Balanced Salt Solution (HBSS) 1X [-calcium, -magnesium, -phenol red]	ThermoFisher Scientific	14175-046	
Bovine Serum Albumin (BSA)	Sigma Aldrich	A2153	Use at 1% concentration for Anti-Human CD56 (NCAM).
DNAase	Sigma Aldrich	DN-25	Use at 0.05% concentration.
Anti-Human CD56 (NCAM) Antibody [Mouse]	BD Pharmingen	555518	Use at 1 : 50.
Propidium iodide	Sigma Aldrich	P4170	Dilute to 1mg/mL in PBS and keep sterile. Use at 1:1000 to achieve a concentration of 10μg/mL.
Reagents for Glass Coverslips			
Autoclaved deionized water			
Alconox detergent	Sigma Aldrich	Z273228	
Ethanol 95%			
Nitric Acid	Sigma Aldrich	438073-M	CAUTION: Concentrated nitric acid is highly corrosive, and its vapours are potentially harmful.
Concentrated hydrochloric acid (HCl)			CAUTION: Concentrated hydrochloric acid is highly corrosive, and its vapours are potentially harmful.
Reagents for Immunocytochemistry			
Paraformaldehyde (PFA)	Merck Millipore	1040051000	Use at a concentration of 4%. CAUTION: PFA is a potent fixative. Avoid ingestion and contact with skin
Triton X-100	Fisher Scientific	10254640	Use at a concentration of 0.1%.
Serum [Donkey]	Merck Millipore	S30-100ML	

Anti-MAP2 Antibody [Chicken]	Abcam	ab5392	Use at a concentration of 1 : 5,000.
Tau HT7 Monoclonal Antibody [Mouse]	ThermoFisher Scientific	MN1000	Use at a concentration of 1 : 500.
Cy3 Anti-Chicken Antibody [Donkey]	Jackson ImmunoResearch	703-165-155	Use at a concentration of 1 : 400.
Alexa Fluor488 Anti-Mouse Antibody [Donkey]	Jackson ImmunoResearch	715-545-150	Use at a concentration of 1 : 400.
4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI)	Sigma Aldrich	D9542	Reconstitute the powder in Milli-Q water to 1 mg/mL. Aliquot and store at -20°C, light sensitive . Use at a concentration of 1 : 500.
Equipment			
T75 flask [Nunc] Delta Surface	ThermoFisher Scientific	156499	
24-well plate [Nunc]	ThermoFisher Scientific	142485	
1.5 mL polypropylene tube	Sigma Aldrich	Z336769	
15 mL falcon tube	Sarstedt	62.554.502	
50 mL falcon tube	Sarstedt	62.547.254	
CryoPure tube 1.6 mL	Sarstedt	72.380	
Pipette controller			For pipetting volumes 1-25 mL.
Sterile serological pipettes: 5, 10 and 25 mL	Sarstedt	86.1253.001, 86.1254.001, 86.1685.001	
Adjustable volume pipettors: 5, 20, 200, and 1,000 µL			
Sterile pipette tips			For pipetting volumes of 0.5 - 1,000 µL.
Glass coverslips	NeuViro	GG-12-1.5-oz	#1.5 thickness, 12mm diameter, 0.5oz, CE certified, fit 24 well plates.
Glass dish			Approximately 150mm diameter.
Glass beaker			Make sure to have an appropriate size beaker for the sonicator bath available. Water from the sonicator bath should not overflow into the glass beaker.
Parafilm M	VWR		
ThawSTAR Automated Cell Thawing System	BioCision	BCS-601	
Countess II Automated Cell Counter	ThermoFisher Scientific	AMQAX1000	
Cell counting chambers [50 slides] and trypan blue [0.4%]	ThermoFisher Scientific	C10228	For use with Countess II Automated Cell Counter.
CoolCell Cell LX Controlled-rate Freezing Container	Biocision	BCS-405	
Laminar flow hood			
Humidified 5% CO ₂ 37 °C incubator			
Centrifuge			Suitable for 1,5, 15 and 50 mL tubes.
Orbital shaker			
Sonicator - Branson Model B200 cleaner	Sigma Aldrich	Z305359	Frequency = 50-60Hz, Amplitude = 30 Watts
FACS Aria III cell sorter	BD Pharmingen		
Phase contrast microscope	Olympus	CKX31	

Inverted fluorescence microscope	Leica	DMI6000 B	
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