

Organotypic Cultures of Adult Human Cortex as an *Ex vivo* Model for Human Stem Cell Transplantation and Validation

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Materials

Name	Company	Catalog Number	Comments
Tissue Cutting and electrophysiology			
Adenosine 5'-triphosphate magnesium salt	Sigma	A9187	
Bath temperature controller	Luigs & Neumann	TC0511354	
Calcium Chloride dihydrate	Merck	102382	
Carbogen gas	Air Liquide	NA	
Cooler	Julaba FL 300	9661012.03	
D-(+)-Glucose	Sigma-Aldrich	G7021	
Double Patch-Clamp amplifier	HEKA electronic	EPC10	
Guanosine 5'-Triphosphate disodium salt	Millipore	371701	
HEPES	AppliChem	A1069	
Magnesium Chloride hexahydrate	Sigma-Aldrich	M2670	
Magnesium Sulfate heptahydrate	Sigma-Aldrich	230391	
Patchmaster	HEKA electronic	Patchmaster 2x91	
Pipette Puller	Sutter	P-2000	
Plastic Petri dish	Any suitable		
Potassium chloride	Merck	104936	
Potassium D-gluconate	ThermoFisher	B25135	
Rubber teat + glass pipette	Any suitable		
Sodium Bicarbonate	Sigma-Aldrich	S5761	
Sodium Chloride	Sigma-Aldrich	S7653	
Sodium dihydrogen phosphate monohydrate	Merck	106346	
Sucrose	Sigma-Aldrich	S7903	

Tissue adhesive: Acryl super glue	Loctite	2062278	
Upright microscope	Olympus	BX51WI	
Vibratome	Leica	VT1200 S	
RINSING SOLUTION			
D-(+)Glucose	Sigma-Aldrich	G7021	
HBSS (without Ca, Mg, or PhenolRed)	ThermoFisher Scientific	14175095	
HEPES	AppliChem	A1069	
Penicillin-Streptomycin (10,000 U/ mL)	ThermoFisher Scientific	15-140-122	
MANTAINANCE AND CULTURE OF HUMAN NEOCORTICAL TISSUE			
6-well plate	ThermoFisher Scientific	140675	
Alvetex scaffold 6 well insert	Reinnervate Ltd	AVP004-96	
B27 Supplement (50x)	ThermoFisher Scientific	17504001	
BrainPhys without Phenol Red	StemCell technologies	#05791	Referenced as neuronal medium in the text
Filter units 250 mL or 500 mL	Corning Sigma	CLS431096/97	
Forceps	Any suitable		
Gentamicin (50 mg/mL)	ThermoFisher Scientific	15750037	
Glutamax Supplement (100x)	ThermoFisher Scientific	35050061	Referenced as L-glutamine in the text
Rubber teat + Glass pipette	Any suitable		
GENERATION OF It-NES cells			
2-Mercaptoethanol 50 mM	ThermoFisher Scientific	31350010	
Animal Free Recombinant EGF	Peprtech	AF-100-15	
B27 Supplement (50x)	Thermo Fisher Scientific	17504001	
bFGF	Peprtech	AF-100-18B	
Bovine Albumin Fraction V (7.5% solution)	ThermoFisher Scientific	15260037	
Cyclopamine, V. calciforicum	Calbiochem	# 239803	
D (+) Glucose solution (45%)	Sigma	G8769	
Dimethyl sulfoxide (DMSO)	Sigma Aldrich	D2438-10mL	
DMEM/F12	ThermoFisher Scientific	11320074	
Dulbecco's Phosphate Buffer Saline (DPBS)	Thermo Fisher Scientific	14190-144	Without calcium and magnesium
Laminin Mouse Protein, Natural	Thermo Fisher Scientific	23017015	
MEM Non-essential aminoacids solutions (100x)	ThermoFisher Scientific	11140050	
N-2 Supplement (100 x)	ThermoFisher Scientific	17502001	
Poly-L-Ornithine	Merk	P3655	
Recombinant Human BMP-4 Protein	R&D Systems	314-BP-010	
Recombinant Human Wnt-3a Protein	R&D Systems	5036-WN	
Sodium Pyruvate (100 mM)	ThermoFisher Scientific	11360070	
Soybean Trypsin Inhibitor, powder	Thermo Fisher Scientific	17075029	
Sterile deionized water	MilliQ		MilliQ filter system
Trypsin EDTA (0.25%)	Sigma	T4049-500ML	
EQUIPMENT FOR CELL CULTURE			
Adjustable volume pipettes 10, 100, 200, 1000 µL	Eppendorf	Various	

Basement membrane matrix ESC-qualified (Matrigel)	Corning	CLS354277-1EA	
Centrifuge	Hettich Centrifugen	Rotina 420R	5% CO ₂ , 37 °C
Incubator	ThermoForma Steri-Cult CO ₂	HEPA Class100	
Stem cell cutting tool 0.190-0.210 mm	Vitrolife	14601	
Sterile tubes	Sarstedt	Various	
Sterile Disposable Glass Pasteur Pipettes 150 mm	VWR	612-1701	
Sterile pipette tips 0.1-1000 µL	Biotix VWR	Various	
Sterile Serological Pipettes 5, 10, 25, 50 mL	Costar	Various	
T25 flasks Nunc	ThermoFisher Scientific	156367	
IMMUNOHISTOCHEMISTRY			
488-conjugated AffinityPure Donkey anti-mouse IgG	Jackson ImmunoReserach	715-545-151	
488-conjugated AffinityPure Donkey anti-rabbit IgG	Jackson ImmunoReserach	711-545-152	
488-conjugated AffinityPure Donkey anti-chicken IgG	Jackson ImmunoReserach	703-545-155	
Alexa fluor 647-conjugated Streptavidin	Jackson ImmunoReserach	016-600-084	
Bovine Serum Albumin	Jackson ImmunoReserach	001-000-162	
Chicken anti-GFP	Merk Millipore	AB16901	
Chicken anti-MAP2	Abcam	ab5392	
Cy3-conjugated AffinityPure Donkey anti-chicken IgG	Jackson ImmunoReserach	703-165-155	
Cy3-conjugated AffinityPure Donkey anti-goat IgG	Jackson ImmunoReserach	705-165-147	
Cy3-conjugated AffinityPure Donkey anti-mouse IgG	Jackson ImmunoReserach	715-165-151	
Diazabicyclooctane (DABCO)	Sigma Aldrich	D27802	Mounting media
Goat anti-AIF1 (C-terminal)	Biorad	AHP2024	
Hoechst 33342	Molecular Probes		Nuclear staining
Mouse anti-MBP	BioLegend	808402	
Mouse anti-SC123	Stem Cells Inc	AB-123-U-050	
Normal Donkey Serum	Merk Millipore	S30-100	
Paint brush	Any suitable		
Paraformaldehyde (PFA)	Sigma Aldrich	150127	
Potassium Phosphate Buffer Saline, KPBS (1x)			
Distilled water			
Potassium dihydrogen Phosphate (KH ₂ PO ₄)	Merk Millipore	104873	
Potassium phosphate dibasic (K ₂ HPO ₄)	Sigma Aldrich	P3786	
Sodium chloride (NaCl)	Sigma Aldrich	S3014	
Rabbit anti-NeuN	Abcam	ab104225	
Rabbit anti-Olig2	Abcam	ab109186	
Rabbit anti-TMEM119	Abcam	ab185333	
Sodium azide	Sigma Aldrich	S2002-5G	
Sodium citrate			

Distilled water			
Tri-Sodium Citrate	Sigma Aldrich	S1804-500G	
Tween-20	Sigma Aldrich	P1379	
Triton X-100	ThermoFisher Scientific	327371000	
EQUIPMENT FOR IMMUNOHISTOCHEMISTRY			
Confocal microscope	Zeiss	LSM 780	
Microscope Slides 76 mm x 26 mm	VWR	630-1985	
Microscope Coverslips 24 mm x 60 mm	Mariefeld	107242	
Microscope Software	Zeiss	ZEN Black edition	
Rubber teat + Glass pipette	Any suitable		