Materials List for:

Isolation, Propagation and Prion Protein Expression During Neuronal Differentiation of Human Dental Pulp Stem Cells

Stefano Martellucci^{1,2}, Costantino Santacroce¹, Valeria Manganelli², Francesca Santilli^{1,2}, Luca Piccoli³, Michele Cassetta³, Roberta Misasi², Maurizio Sorice², Vincenzo Mattei^{1,2}

Correspondence to: Vincenzo Mattei at v.mattei@sabinauniversitas.it

URL: https://www.jove.com/video/59282

DOI: doi:10.3791/59282

Materials

Name	Company	Catalog Number	Comments
Amphotericin B solution	Sigma-Aldrich	A2942	It is use to supplement cell culture media, it is a polyene antifungal antibiotic from Streptomyce
Anti-B3tubulin	Cell Signaling Technology	#4466	One of six B-tubulin isoform, it is expressed highly during fetal and postnatal development, remaining high in the peripheral nervous system
Anti-CD105	BD Biosciences	611314	Endoglin (CD105), a major glycoprotein of human vascular endothelium, is a type I integral membrane protein with a large extracellular region, a hydrophobic transmembrane region, and a short cytoplasmic tail
Anti-CD44	Millipore	CBL154-20ul	Positive cell markers antibodies directed against mesenchymal stem cells
Anti-CD73	Cell Signaling Technology	13160	CD73 is a 70 kDa glycosyl phosphatidylinositol-anchored, membrane-bound glycoprotein that catalyzes the hydrolysis of extracellular nucleoside monophosphates into bioactive nucleosides
Anti-CD90	Millipore	CBL415-20ul	Positive cell markers antibodies directed against mesenchymal stem cells
Anti-GAP43	Cell Signaling Technology	#8945	Is a nervous system specific, growth-associated protein in growth cones and areas of high plasticity
Anti-mouse PE	Abcam	ab7003	Is an antibody used in in flow cytometry or FACS analysis
Anti-NFH	Cell Signaling Technology	#2836	Is an antibody that detects endogenous levels of total Neurofilament-H protein
Anti-PrP mAb EP1802Y	Abcam	ab52604	Rabbit monoclonal [EP 1802Y] to Prion protein PrP
Anti-rabbit CY5	Abcam	ab6564	Is an antibody used in in flow cytometry or FACS analysis

¹Laboratory of Experimental Medicine and Environmental Pathology - Rieti University Hub, "Sabina Universitas"

²Department of Experimental Medicine, "Sapienza" University

³Department of Science Dentistry and Maxillofacial, "Sapienza" University

	·	_	
Anti-STRO 1	Millipore	MAB4315-20ul	Positive cell markers antibodies directed against mesenchymal stem cells
B27 Supp XF CTS	Gibco by life technologies	A14867-01	B-27 can be used to support induction of human neural stem cells (hNSCs) from pluripotent stem cells (PSCs), expansion of hNSCs, differentiation of hNSCs, and maintenance of mature differentiated neurons in culture
BD Accuri C6 flow cytometer	BD Biosciences	AC6531180187	Flow cytometer equipped with a blue laser (488 nm) and a red laser (640 nm)
BD Accuri C6 Software	BD Biosciences		Controls the BD Accuri C6 flow cytometer system in order to acquire data, generate statistics, and analyze results
bFGF	PeproThec, DBA	100-18B	basic Fibroblast Growth Factor
Centrifuge CL30R	Termo fisher Scientific	11210908	it is a device that is used for the separation of fluids,gas or liquid, based on density
CO2 Incubator 3541	Termo fisher Scientific	317527-185	it ensures optimal and reproducible growth conditions for cell cultures
Collagenase, type IV	Life Technologies	17104019	Collagenase is a protease that cleaves the bond between a neutral amino acid (X) and glycine in the sequence Pro-X-Glyc-Pro, which is found with high frequency in collagen
Disposable scalpel	Swann-Morton	501	It is use to cut tissues
DMEM-L	Euroclone	ECM0060L	Dulbecco's Modified Eagle's Medium Low Glucose with L- Glutamine with Sodium Pyruvate
EGF	PeproThec, DBA	AF-100-15	Epidermal Growth Factor
Fetal Bovine Serum	Gibco by life technologies	10270-106	FBS is a popular media supplement because it provides a wide array of functions in cell culture. FBS delivers nutrients, growth and attachment factors and protects cells from oxidative damage and apoptosis by mechanisms that are difficult to reproduce in serum-free media (SFM) systems
Filtropur BT50 0.2,500ml Bottle top filter	Sarstedt	831,823,101	it is a device that is used for filtration of solutions
Flexitube GeneSolution for PRNP	Qiagen	GS5621	4 siRNAs for Entrez gene 5621. Target sequence N.1 TAGAGATTTCATAGCTATTTA N.2 CAGCAAATAACCATTGGTTAA N.3. CTGAATCGTTTCATGTAAGAA N.4 CAGTGACTATGAGGACCGTTA
Hank's solution 1x	Gibco by life technologies	240200083	The essential function of Hanks 'Balanced Salt solution is to maintain pH as well as osmotic balance. It also provides water and essential inorganic ions to cells
HiPerFect Transfection Reagent	Qiagen	301705	HiPerFect Transfection Reagent is a unique blend of cationic and

			neutral lipids that enables effective siRNA uptake and efficient release of siRNA inside cells, resulting in high gene knockdown even when using low siRNA concentrations
Neurobasal A	Gibco by life technologies	10888022	Neurobasal-A Medium is a basal medium designed for long-term maintenance and maturation of pure post-natal and adult brain neurons
Paraformaldehyde	Sigma-Aldrich	30525-89-4	Paraformaldehyde has been used for fixing of cells and tissue sections during staining procedures
penicillin/streptomycin	Euroclone	ECB3001D	It is use to supplement cell culture media to control bacterial contamination
Phosphate buffered saline (PBS)	Euroclone	ECB4004LX10	PBS is a balanced salt solution used for the handling and culturing of mammalian cells. PBS is used to to irrigate, wash, and dilute mammalian cells. Phosphate buffering maintains the pH in the physiological range
TC-Platte 6 well, Cell+,F	Sarstedt	833,920,300	It is a growth surface for adherent cells
Tissue culture flask T-25,Cell +,Vented Cap	Sarstedt	833,910,302	Tissue culture flask T-25, polystyrene, Cell+ growth surface for sensitive adherent cells, e.g. primary cells, canted neck, ventilation cap, yellow, sterile, Pyrogen-free, non-cytotoxic, 10 pcs./bag
Triton X-100	Sigma-Aldrich	9002-93-1	Widely used non-ionic surfactant for recovery of membrane components under mild non-denaturing conditions
Trypsin-EDTA	Euroclone	ECB3052D	Trypsin will cleave peptides on the C-terminal side of lysine and arginine amino acid residues. Trypsin is used to remove adherent cells from a culture surface
Tube	Sarstedt	62,554,502	Tube 15ml, 120x17mm, PP
VBH 36 C2 Compact	Steril	ST-003009000	Offers totally protection for the enviroment and worker
ZEISS Axio Vert.A1 – Inverted Microscope	Zeiss	3849000962	ZEISS Axio Vert.A1 provides a unique entry level price and can provide all contrasting techniques, including brightfield, phase contrast, PlasDIC, VAREL, improved Hoffman Modulation Contrast (iHMC), DIC and fluorescence. Incorporate LED illumination for gentle imaging for fluorescently-labeled cells. Axio Vert.A1 is ergonomically designed for routine work and compact enough to sit inside tissue culture hoods.