

Materials List for:

Post-differentiation Replating of Human Pluripotent Stem Cell-derived Neurons for High-Content Screening of Neuritogenesis and Synapse Maturation

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Materials

Name	Company	Catalog Number	Comments
Post Replating Media			
L-Ascorbic Acid	Sigma	A4403	Add 1ml of 200mM stock to 1L of N2B27 media
dibutyryl-cAMP	Sigma	D0627	Add 1 µM
Human BDNF	Peprtech	450-02	10 ng/ml final concentration
B27 (50X)	Thermofisher Scientific	17504044	Add 20 ml to 1L N2B27 media
DMEM/F12 with Glutamax	Thermofisher Scientific	31331093	Add N2 and distribute in 50 mL conicals; parafilm wrap lids
Human GDNF	Peprtech	450-10	10 ng/ml final concentration
Glutamax	Thermofisher Scientific	35050038	Add 10 ml to 1L N2B27 media; glutamine supplement
Mouse Laminin	Sigma	P3655-10mg	Add 100 µl to 50 mL N2B27
MEM Nonessential Amino Acids	Thermofisher Scientific	11140035	Add 5ml to 1L N2B27 media
N2 (100X) Supplement	Life Technologies	17502048	Add 5ml to 500mL media
Neurobasal A Media	Thermofisher Scientific	10888022	Combine with DMEM/F12 to generate N2B27 media for CVB wt cells; neural basal A media
Neurobasal Media	Thermofisher Scientific	21103049	for WT126 cells; neural basal media
SM1 Supplement	StemCell Technologies	5711	Add 1:50 to media
sodium bicarbonate	Thermofisher Scientific	25080-094	Add 10ml to 1L N2B27 media
Plate Preparation			
10cm Tissue Culture Dishes	Fisher Scientific	08772-E	Plastic TC-treated dishes
6-well Tissue Culture Dishes	Thomas Scientific	1194Y80	NEST plates
Mouse Laminin	Life Technologies	23017-015	Add 1:400 on plastic
Poly-Ornithine	Sigma	P3655-10mg	Add 1:1000 on plastic
UltraPure Distilled Water	Life Technologies	10977-015	To dilute Poly-L-Ornithine
Replating Reagents			
100mM Cell Strainer	Corning	431752	Sterile, individually wrapped
384-well plate, uncoated	PerkinElmer	6007550	Coat with PLO and Laminin
DPBS	Life Technologies	14190144	Dulbecco's phosphate-buffered saline
Poly-D-Lysine-Precoated 384-well Plates	PerkinElmer	6057500	Rinse before coating with laminin

StemPro Accutase	Life Technologies	A1110501	Apply 1mL/10cm plate for 30-45 minutes; proteolytic enzyme
Fixation Materials			
37% Formaldehyde	Fisher Scientific	F79-1	Dissolved in PBS
Sucrose	Fisher Scientific	S5-12	0.8 g per 10 ml of fixative
Immunostaining Materials			
Alexa Fluor 488 Goat anti-mouse	Invitrogen	A-11001	secondary antibody
Alexa Fluor 568 Goat anti-chicken	Invitrogen	A-11041	secondary antibody
Alexa Fluor 647 Goat anti-chicken	Invitrogen	A-21449	secondary antibody
Alexa Fluor 561 Goat anti-rat	Invitrogen	A-11077	secondary antibody
DAPI	Biotium	40043	visualizes DNA
mouse antibody against b3-tubulin (TuJ-1)	Neuromics	MO15013	early stage neuronal marker
rat antibody against CTIP2	Abcam	ab18465	layer 5/6 cortical neurons
chicken antibody against MAP2	LifeSpan Biosciences	LS-B290	early stage neuronal marker
chicken antibody against NeuN	Millipore	ABN91	late stage neuronal marker
rabbit antibody against MAP2	Shelley Halpain	N/A	early stage neuronal marker
mouse antibody against PSD-95	Sigma	P-246	post-synaptic marker
rabbit antibody against Synapsin 1	Millipore	AB1543	pre-synaptic marker
Bovine serum albumin (BSA)	GE Healthcare Life Sciences	SH30574.02	10% in PBS for blocking
Titon X-100	Sigma	9002931	Dilute to 0.2% on PBS for permeabilization
Viability Markers			
Vivafix 649/660	Biorad	135-1118	cell death marker
Calcium Imaging			
Name of Reagent/ Equipment	Company	Catalog Number	Comments/Description
AAV8-syn-jGCAMP7f-WPRE	THE SALK INSTITUTE, GT3 Core Facility	N/A	calcium reporter in a viral delivery system
hiPSC-derived NPCs			
WT 126 (Y2610)	Gage lab	N/A	Marchetto et al., 2010
CVB WT24	Yeo and Goldstein labs	N/A	unpublished