

Materials List for:

Protocol for the Differentiation of Human Induced Pluripotent Stem Cells into Mixed Cultures of Neurons and Glia for Neurotoxicity Testing

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Materials

Name	Company	Catalog Number	Comments
Complete hiPSC medium:			
mTeSR1 Basal Medium	Stem Cell Technologies	05851	(Step 1.2.6). Complete mTeSR1 is stable when stored at 2 - 8 °C for up to 2 weeks. 5x Supplements can be dispensed into working aliquots and stored at -20 °C. Use frozen aliquots within 3 months.
mTeSR1 5x Supplements	Stem Cell Technologies	05852	
Matrigel hESC-qualified Matrix	Corning	354277	1:100 (Step 1.1). Thaw Matrigel on ice, prepare 200 µL aliquots and store them in -80 °C. For coating, dilute 200 µL aliquot in 20 mL of DMEM/F12 medium.
CryoStem Freezing Medium	Stemgent	01-0013-50	Freeze ~ 100 fragments/250 µL/vial (Step 1.2.1)
Name	Company	Catalog Number	Comments
hiPSC EB medium:			
Knockout DMEM	Thermo-Fisher	10829-018	(Step 2.1.7)
Knockout Serum Replacement (KOSR)	Thermo-Fisher	10828-028	20% final concentration (Step 2.1.7)
Non-Essential Amino Acids	Thermo-Fisher	11140-035	(Step 2.1.7)
Penicillin/Streptomycin	Thermo-Fisher	15140-122	50 U/mL final concentration (Step 2.1.7)
L-Glutamine 200 mM Solution	Thermo-Fisher	25030-081	2 mM final concentration (Step 2.1.7)
β-Mercaptoethanol	Thermo-Fisher	31350-010	50 µM final concentration (Step 2.1.7)
Name	Company	Catalog Number	Comments
Complete neuroepithelial induction medium (NRI):			
DMEM/F12	Thermo-Fisher	3133-038	(Step 2.3.1)
Non-Essential Amino Acids	Thermo-Fisher	11140-035	(Step 2.3.1)
N2 Supplement	Thermo-Fisher	17502-048	(Step 2.3.1)
Penicillin/Streptomycin	Thermo-Fisher	15140-122	50 U/mL final concentration (Step 2.3.1)
Heparin Grade I-A, ≥180 USP units/mg	Sigma-Aldrich	H3149-100KU	2 µg/mL final concentration (Step 2.3.1)
bFGF	Thermo-Fisher	13256-029	20 ng/mL final concentration added before use (Step 2.3.1)
Matrigel Basement Membrane Matrix	Corning	354234	1:100 (Step 2.2). Thaw Matrigel on ice, prepare 200 µL aliquots and

			store them in -80 °C. For coating, dilute 200 µL aliquot in 20 mL of cold DMEM/F12 medium.
Laminin	Sigma-Aldrich	L2020	1:100 (Step 2.2). Dilute in PBS 1x.
Name	Company	Catalog Number	Comments
Complete Neuronal Differentiation medium (ND):			
Neurobasal Medium	Thermo-Fisher	21103049	(Step 2.4.11)
B-27 Supplements (50x)	Thermo-Fisher	17504044	(Step 2.4.11)
N2 Supplement	Thermo-Fisher	17502-048	(Step 2.4.11)
Penicillin/Streptomycin	Thermo-Fisher	15140-122	50 U/mL final concentration (Step 2.4.11)
GDNF	Thermo-Fisher	PHC7045	1 ng/mL final concentration. Added before use. (Step 2.4.11)
BDNF	Thermo-Fisher	PHC7074	2.5 ng/mL final concentration. Added before use. (Step 2.4.11)
Name	Company	Catalog Number	Comments
Neural induction medium (NI):			
DMEM/F12	Thermo-Fisher	3133-038	(Step 3.3)
Non-Essential Amino Acids	Thermo-Fisher	11140-035	(Step 3.3)
N2 Supplement	Thermo-Fisher	17502-048	(Step 3.3)
Penicillin/Streptomycin	Thermo-Fisher	15140-122	50 U/mL final concentration (Step 3.3)
Heparin Grade I-A, ≥180 USP units/mg	Sigma-Aldrich	H3149-100KU	2 µg/mL final concentration (Step 3.3)
B-27 Supplement (50x), minus vitamin A	Thermo-Fisher	12587010	(Step 3.3)
L-Glutamine 200 mM Solution	Thermo-Fisher	25030-081	2 mM final concentration (Step 3.3)
bFGF	Thermo-Fisher	13256-029	10 ng/mL final concentration. Added before use (Step 3.3)
EGF	Thermo-Fisher	PHG6045	10 ng/mL final concentration. Added before use (Step 3.3)
BDNF	Thermo-Fisher	PHC7074	2.5 ng/mL final concentration. Added before use (Step 3.3)
Defined Trypsin Inhibitor (DTI)	Thermo-Fisher	R007-100	Pre-warm at 37 °C. Add an equal amount of DTI to Trypsin-EDTA (Step 3.10)
Trypsin-EDTA (0.5%), no phenol red	Thermo-Fisher	15400054	1:10. Dilute Trypsin-EDTA in PBS 1x (without calcium and magnesium), pre-warm the solution at 37 °C (Step 3.8)
CryoStor cell cryopreservation medium	Sigma-Aldrich	C2874-100ML	(Step 4.2)
Trypan Blue (0.4%)	Sigma-Aldrich	T8154-100ML	multiple manufacturers/suppliers
Name	Company	Catalog Number	Comments
TaqMan Probesets and reagents for gene expression analysis:			
RNAqueous-Micro kit	Thermo-Fisher	AM1931	(Step 5.1.6)
High Capacity cDNA Reverse Transcription Kits	Thermo-Fisher	4368814	
TaqMan Gene Expression Master Mix	Thermo-Fisher	4369016	
GFAP	Thermo-Fisher	Hs00909233_m1	

MAP2	Thermo-Fisher	Hs00258900_m1	
NQO1	Thermo-Fisher	Hs02512143_s1	
SRXN1	Thermo-Fisher	Hs00607800_m1	
HMOX1	Thermo-Fisher	Hs01110250_m1	
GSR	Thermo-Fisher	Hs00167317_m1	
PAX6	Thermo-Fisher	Hs01088112_m1	
NES	Thermo-Fisher	Hs00707120_s1	
GRIA1	Thermo-Fisher	Hs00181348_m1	
GAP43	Thermo-Fisher	Hs00967138_m1	
GABRA3	Thermo-Fisher	Hs00968132_m1	
GABRA1	Thermo-Fisher	Hs00168058_m1	
NR4A2	Thermo-Fisher	Hs00428691_m1	
TH	Thermo-Fisher	Hs00165941_m1	
GAPDH	Thermo-Fisher	Hs02758991_g1	
ACTB	Thermo-Fisher	Hs99999903_m1	
MAPT	Thermo-Fisher	Hs00902194_m1	
SYP	Thermo-Fisher	Hs00300531_m1	
NANOG	Thermo-Fisher	Hs04260366_g1	
POU5F1 (OCT4)	Thermo-Fisher	Hs04195369_s1	
SOX1	Thermo-Fisher	Hs01057642_s1	
AFP	Thermo-Fisher	Hs00173490_m1	
KRT18	Thermo-Fisher	Hs01941416_g1	
NPPA	Thermo-Fisher	Hs00383230_g1	
T	Thermo-Fisher	Hs00610080_m1	
NCAM1	Thermo-Fisher	Hs00941821_m1	
NR4A1	Thermo-Fisher	Hs00374226_m1	
PHOX2A	Thermo-Fisher	Hs00605931_mH	
PHOX2B	Thermo-Fisher	Hs00243679_m1	
NARG2	Thermo-Fisher	Hs00973298_g1	
SLC18A3	Thermo-Fisher	Hs00268179_s1	
SLC5A7	Thermo-Fisher	Hs00222367_m1	
ISL1	Thermo-Fisher	Hs00158126_m1	
LHX3	Thermo-Fisher	Hs01033412_m1	
TaqMan Human Protein Kinase Array	Thermo-Fisher	4418721	
Name	Company	Catalog Number	Comments
Antibodies and reagents for immunostaining:			
B-III-tubulin (Tuj1)	Covance	MMS-435P	1:500 (Step 5.2.5). Other antibodies may also be used.
MAP2	Sigma Aldrich	M4403	1:500
NF200	Sigma Aldrich	N4142	1:1000
GFAP	Acris Antibodies GmbH	AP02002SU-N	1:500
Nestin	Sigma-Aldrich	N5413	1:200
synaptophysin (SYN)	Abcam	AB14692	1:200
Tau	Thermo-Fisher	MA5-12808	1:100
Nrf2	Abcam	AB62352	1:200

Keap1	Abcam	AB66620	1:200
sulfiredoxin1 (SRXN1)	Abcam	AB92298	1:200
NAD(P)H quinone oxidoreductase 1 (NQO1)	Abcam	AB2346	1:200
OCT4	Millipore	MAB4401	1:100
SSEA3	Millipore	MAB4303	1:100
Tra1-60	Millipore	MAB4360	1:250
Tyrosine hydroxylase (TH)	Millipore	AB152	1:200
Gamma-aminobutyric acid (GABA)	Sigma-Aldrich	A0310	1:100
Vesicular glutamate transporter 1 (VGlut1)	Abcam	AB72311	1:500
Paraformaldehyde	Sigma-Aldrich	P6148-500G	4% (4% formaldehyde can also be used)
DPBS, no calcium, no magnesium	Thermo-Fisher	14190144	
Triton-X-100 Solution	Sigma-Aldrich	93443-100ML	0.1%
BSA 35%	Sigma-Aldrich	A7979-50ML	3.5%
Donkey anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 594 conjugate	Thermo-Fisher	SA5-10040	1:500. (Step 5.2.7) Other fluorochrome-conjugated secondary antibodies may also be used. In this case, appropriate dilutions should be tested by the enduser.
Donkey anti-Mouse IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate	Thermo-Fisher	SA5-10166	1:500
Donkey anti-Goat IgG (H+L) Cross Adsorbed Secondary Antibody, DyLight 488 conjugate	Thermo-Fisher	SA5-10086	1:500
DAPI Solution (1 mg/mL)	Thermo-Fisher	62248	1:1000 (Step 5.2.7)
Name	Company	Catalog Number	Comments
Antibodies for Reverse Phase Protein Array (RPPA):			
4E-BP1 (S65)	Abcam	AB81297	1:250 (Note after step 5.2.8)
Akt (T308)	Cell Signaling	9275	1:100
Akt (S473)	Cell Signaling	9271	1:100
AMPKalpha (T172)	Cell Signaling	2531	1:100
AMPKbeta1 (S108)	Cell Signaling	4181	1:100
ATF-2 (T71)	Cell Signaling	9221	1:100
c-Jun (S63)	Cell Signaling	9261	1:200
c-Jun (S73)	Cell Signaling	9164	1:200
c-Kit (Y719)	Cell Signaling	3391	1:250
CREB (S133)	Cell Signaling	9191	1:100
EGFR (Y1068)	Cell Signaling	2234	1:50
ErbB2/HER2 (Y1248)	Cell Signaling	2247	1:100
ERK 1/2, p44/42 (T202/Y204)	Cell Signaling	9101	1:2000
GSK-3alpha (S21)	Cell Signaling	9337	1:50
Jak1 (Y1022/1023)	Cell Signaling	3331	1:100
Lck (Y505)	Cell Signaling	2751	1:500
LKB1 (S428)	Cell Signaling	3051	1:100
mTOR (S2448)	Cell Signaling	5536	1:100

NFkB p65 (S536)	Cell Signaling	3031	1:50
p70 S6 Kinase (T389)	Cell Signaling	9205	1:200
PDK1 (S241)	Cell Signaling	3061	1:100
PKA C (T197)	Cell Signaling	4781	1:250
PRAS40 (T246)	BioSource	44-1100	1:2000
PTEN (S380)	Cell Signaling	9551	1:500
Smad1 (S463/465), Smad5 (S463/465), Smad8 (S426/428)	Cell Signaling	9511	1:500
Src (Y527)	Cell Signaling	2105	1:500
Src Family (Y416)	Cell Signaling	2101	1:200
Stat1 (Y701)	Cell Signaling	9171	1:200
Stat3 (S727)	Cell Signaling	9134	1:200
Zap-70 (Y319)	Enogene	E011159	1:100
β Catenin (S33/37/T41)	Cell Signaling	9561	1:250
CREB	Upstate Biotechnologies	06-863	1:100
Fos B	Cell Signaling	2251	1:200
GRB2	Cell Signaling	3972	1:2000
HSP70	Stressgen	SPA-810	1:100
c-Jun	Cell Signaling	9165	1:100
Kip1/p27	BD	610241	1:100
Lck	Cell Signaling	2984	1:250
Mcl-1	Cell Signaling	4572	1:80
Musashi	Cell Signaling	2154	1:100
NOTCH1	Cell Signaling	3439	1:100
PTEN	Cell Signaling	9552	1:500
SGK1	Abnova	PAB4590	1:250
Zap-70	Cell Signaling	2705	1:250
β -Catenin	Abcam	AB32572	1:1000
Dll4	Abcam	AB7280	1:500
Shh	Abcam	AB53281	1:250
HIF-1 α	BD	610958	1:50
NUMB PAN-ISO	Upstate Biotechnologies	07-207	1:400
NUMB-L	Chemicon	AB15145	1:750
Cyclin B	BD	610220	1:75
c-Myc	Calbiochem	OP-10	1:100
BCIP/NBT Kit	Thermo-Fisher	002209	(Note after step 5.2.8). Kit used to measure alkaline phosphatase activity, similar kits can be used.
Name	Company	Catalog Number	Comments
Antibodies for Flow Cytometry:			
SSEA1 Antibody, Pacific Blue conjugate	Thermo-Fisher	MHCD1528	1:100 (Note after step 5.2.8)
SSEA4 Antibody (MC813-70), Alexa Fluor 647	Thermo-Fisher	SSEA421	1:100
Name	Company	Catalog number	Comments
Specific instruments, tools and softwares:			

Countess Automated Cell Counter	Thermo-Fisher	C10227	Neubauer chamber or other suitable glass hemocytometer can be used.
MEA1060-Inv-BC	Multichannel Systems	MEA1060-Inv-BC	(Step 5.3)
MEA1060-BC control software	Multichannel Systems	MEA1060-BC	(Step 5.3)
NeuroExplorer	Multichannel Systems	NeuroExplorer (NE)	(Step 5.3) For post-processing of MEA data
Multielectrode arrays (MEA)	Multichannel Systems	60MEA100/10iR-Ti-gr	(Step 5.3) Single-well MEA chip
ArrayScan XTI High Content Platform	Thermo-Fisher	ASN00002P	(Step 5.2.8) Mean fluorescence can be quantified by using specific ArrayScan algorithms (e.g., Cytotoxicity V.4 and NucTrans V.4 bioapplications). It is recommended to take minimum 20 pictures/well, and have 7-8 internal replicates per condition
7900HT Fast Real-Time PCR System	Thermo-Fisher	4351405	(Step 5.1.6)
BD ULTRA-FINE Needle Insulin Syringe (with 30G needle)	BD	328280	(Steps 1.3.1, 2.1.2, and 2.4.1)
StemPro EZPassage Disposable Stem Cell Passaging Tool	ThermoFisher	23181010	This colony cutting tool can be used as an alternative to the use of 30G needle 1 mL syringes (Step 1.3.1)
Ultra-Low attachment Petri dish (60 mm)	Corning	10010582	(Step 2.1.8) Also other brands can be used.
Mr. Frosty Freezing container	Sigma-Aldrich	C1562-1EA	