

Efficient and Scalable Generation of Human Ventral Midbrain Astrocytes from Human-Induced Pluripotent Stem Cells

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Materials

Name	Company	Catalog Number	Comments
Reagents			
0.2M Tris-Cl (pH 8.5)	n/a	n/a	Made up from Tris base and plus HCl
0.5M EDTA, PH 8	ThermoFisher	15575-020	1:1000 in D-PBS to 0.5 mM final
1,4-diazabicyclo[2.2.2]octane (DABCO)	Sigma	D27802-	25 mg/mL in Mowiol mounting solution
13 mm coverslips	VWR	631-0149	
2-Mercaptoethanol (50 mM)	ThermoFisher	31350010	
Accutase	ThermoFisher	13151014	
Advanced DMEM/F12	ThermoFisher	12634010	Has 1x NEAA but we add to final concentration of 2x (0.2 mM)
Ascorbic acid	Sigma	A5960	200 mM stock, 1:1000 to 200 µM final
B27 Supplement	ThermoFisher	17504-044	50x stock
BSA	Sigma	5470	
Cell freezing media	Sigma	C2874	Cryostor CS10
Cell freezing vessel	Nalgene	5100-0001	
CHIR99021	Axon Medchem	1386	0.8 mM stock, 1:1000 dilution to 0.8 µM final
Cryovials	Sigma	CLS430487	
DAPI	Sigma	D9542	1 mg/mL, 1:10,000 to 100ng/mL final (in PBS)
DMEM/F12 + Glutamax	ThermoFisher	10565018	
Dulbeccos-PBS (D-PBS without Mg or Ca)	ThermoFisher	14190144	pH 7.2
E8 Flex medium kit	ThermoFisher	A2858501	

Formaldehyde (36% solution)	Sigma	47608	
Geltrex	ThermoFisher	A1413302	1:100 or 1:400 in ice-cold DMEM/F12
Glutamax	ThermoFisher	35050038	2 mM stock (1:200 in N2B27, 1:100 in ASTRO media to 20 µM final)
Glycerol	Sigma	G5516	
Human BDNF	Peprtech	450-02	20 µg/mL stock, 1:1000 to 20 ng/mL final
Human BMP4	Peprtech	120-05	20 µg/mL stock, 1:1000 to 20 ng/mL final
Human EGF	Peprtech	AF-100-15	20 µg/mL stock, 1:1000 to 20 ng/mL final
Human GDNF	Peprtech	450-10	20 µg/mL stock, 1:1000 to 20 ng/mL final
Human insulin solution	Sigma	I9278	10 mg/mL stock, 1:2000 to 5 µg/mL final
Human LIF	Peprtech	300-05	20 µg/mL stock, 1:1000 to 20 ng/mL final
IL-6 ELISA kit	Biotechne	DY206	
Isopropanol	Sigma	I9516-4L	For filling Mr Frosty cryostorage vessel
LDN193189	Sigma	SML0559	100 µM stock, 1:10,000 dilution to 10 nM final
Mowiol 40-88	Sigma	324590	
N2 Supplement	ThermoFisher	17502048	100x stock
NEAA	ThermoFisher	11140035	10 mM stock, 1:100 to 0.1 mM final
Neurobasal media	ThermoFisher	21103049	
Normal Goat serum	Vector Labs	S-1000-20	
Revitacell	ThermoFisher	A2644501	100x stock, 1:100 to 1x final
SB431542	Tocris	1614	10 mM stock, 1:1000 dilution to 10 µM final
SHH-C24ii	Biotechne	1845-SH-025	200 µg/mL stock, 1:1000 to 200 ng/mL final
Tris-HCl	Sigma	PHG0002	
Triton-X	Sigma	X100	
Tween-20	Sigma	P7949	
Vitronectin	ThermoFisher	A14700	1:50 in D-PBS
Antibodies for immunocytochemistry	Company	Catalogue Number	Host species
Antibody against S100b	Sigma	SAB4200671	Mouse; 1:200
Antibody against FOXA2	SCBT	NB600501	Mouse; 1:50
Antibody against LMX1A	ProSci	7087	Rabbit; 1:300
Antibody against LMX1A	Millipore	AB10533	Rabbit; 1:2000
Antibody against LMX1B	Proteintech	18278-1-AP	Rabbit; 1:300
Antibody against GLAST	Proteintech	20785-1-AP	Rabbit; 1:300
Antibody against GFAP	Dako	Z0334	Rabbit; 1:400
Antibody against CD49f	Proteintech	27189-1-AP	Rabbit; 1:100
Antibody against MSI1	Abcam	ab52865	Rabbit; 1:400
Alexa Fluor 488 Goat Anti-Rabbit	ThermoFisher	A32731	Goat; 1:500
Alexa Fluor 488 Goat Anti-Mouse	ThermoFisher	A32723	Goat; 1:500
Alexa Fluor 568 Goat Anti-Rabbit	ThermoFisher	A11036	Goat; 1:500
Alexa Fluor 488 Goat Anti-Mouse	ThermoFisher	A11031	Goat; 1:500