May 01, 2024

Differentiation of hPSCs into Dopamine neurons

DOI
https://dx.doi.org/10.17504/protocols.io.q26g71849gwz/v1

Beatrice Weykopf[1,2]

[1]1Stephen and Denise Adams Center for Parkinson's Disease Research of Yale School of Medicine, New Haven, CT 06511, USA;
[2]Aligning Science Across Parkinson's Collaborative Research Network, Chevy Chase, MD 20815, USA

Beatrice Weykopf
Yale

DOI: https://dx.doi.org/10.17504/protocols.io.q26g71849gwz/v1

Protocol Citation: Beatrice Weykopf 2024. Differentiation of hPSCs into Dopamine neurons. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g71849gwz/v1

Protocol status: Working
We use this protocol and it's working

Created: April 22, 2024

Last Modified: May 31, 2024

Protocol Integer ID: 98607

Keywords: ASAPCRN, dopaminergic neuron, dopamine neurons this protocol, dopamine neuron, differentiation

Abstract

This protocol describes the differentiation of hPSCs into dopaminergic neurons modified from Kriks et al., 2011 and Ryan et al., 2013

Materials

SRM Medium

79%	Knockout DMEM
20%	KOSR
1%	L-GlutaMAX
1%	Pen/Strep

Dopa Mat Basal medium

100%	Neurobasal
1x	B27 w/o vitamin A
1%	L-GlutaMAX
1%	Pen/Strep

Dopa Mat Medium

100%	Dopa mat basal medium
20 ng/ml	BDNF
20 ng/ml	GDNF
10 µM	DAPT
221 µM	LAAP
0.5 mM	dbcAMP
1 ng/ml	TGF-ß-III

	Reagent	Concentration	Vendor
	Y-27632	10µM	Stem cell Technologies #72304
	TGFß-III	2 µg/ml	Peprotech #100-36E
	Shh (C25II)	100 ng/ml	R&D Systems #464-SH-200
	SB431542	10 µM	Stemcell Technologies #72232
	Purmorphamine	2 µM	Emdmillipore #540220-5MG
	Poly-D-Lysine hydrobromide	100 µg/ml	Sigma-Aldrich #P1024-50MG
	Matrigel	1:20 - 1:30	Corning #354234
	LDN-1931189	200 nM	Stemgent #04-0046
	Laminin	10µg/ml	R&D Systems #3400-010-02
	LAAP	221 µM	Sigma-Aldrich #A8960-5G

	Reagent	Concentration	Vendor
	GDNF	10 µg/ml	Peprotech #450-10
	FGF-8b	100 µg/ml	Peprotech #100-18B-50UG
	dbcAMP	0.5 mM	Enzo BML-CN125-0100
	DAPT	10 µM	Tocris #2634
	CHIR99021	3 µM	Milteniy Biotec #130-103-926
	AraC Cytosine ß-D-arabinofuranoside	3µM	Sigma-Aldrich #C1768
	Accutase	1x	Gibco #A1110501
	mTeSR plus	1x	Stemcell Technologies #100-027
	Knockout Serum Replacement	n/a	Gibco #10828028
	KnockOut DMEM	n/a	Gibco #10829018
	Neurobasal	n/a	Gibco #21103049
	B27 w/o vitamin A	n/a	Gibco #12587010
	L-GlutaMAX	n/a	Gibco #35050
	Penicillin-Streptomycin	n/a	Gibco #15140122

1 Day -1: seed 200K cell/cm^2 in mTeSR$^+$ medium supplemented with 10µM Rock inhibitor (RI) onto Martigel coated TC plates

2 Day 0: Differentiation initiation. Aspirate mTeSR$^+$ medium and add SRM media supplemented with 200nM LDN193189 + 10µM SB431542

3 Day 1: SRM + LDN + SB supplemented with 100 ng/ml FGF8b, 100 ng/ml ShhC25II and 2µM Purmorphamine

4 Day 2: SRM + LDN + SB+ FGF8b + ShhC25II + Pur

5 Day 3: SRM + LDN + SB+ FGF8b + ShhC25II + Pur +3 µM CHIR99021

6 Day 4: no feed if medium is not consumed

7 Day 5: 75% SRM + 25% Neurobasal + LDN + SB+ FGF8b + ShhC25II + Pur +CHIR

8 Day 6: no feed if medium is not consumed

9 Day 7: 50% SRM + 50% Neurobasal + LDN + CHIR

10 Day 8: no feed if medium is not consumed

11 Day 9: 25% SRM + 75% Neurobasal + LDN + CHIR

12 Day 10: no feed if medium is not consumed

13 Day 11: 100% Dopa mat basal medium + CHIR

14 Day 12: no feed

15 Day 13: Passage cells in a ratio of 1:1 onto matrigel-coated dishes with 30-60min Accutase treatment. Spin down the cells in Dopa mat basal and resuspend in Dopa mat medium supplemented with 10µM Y27632 and CHIR.

16 Day 20-24: Cells are plated on Poly-D-lysine and laminin coated plates in a density of 2-2.5M cells / 6 well. Cells are dissociated using 60-90min Accutase supplemented with 10µM Y27632.

17 Day 25-27: 3µM cytosine arabinoside is added to Dopa mat media. Day 27 cells are washed twice with Dopa mat basal medium to remove any cytosine arabinoside residues.

18 Around Day 30: Cells are dissociated using Accutase supplemented with 10µM Y27632 and plated in onto Poly-D-lysine and laminin coated dishes. The final density depends on the assay.

Protocol references

Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z, Carrillo-Reid L, Auyeung G, Antonacci C, Buch A, Yang L, Beal MF, Surmeier DJ, Kordower JH, Tabar V, Studer L. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature. 2011 Nov 6;480(7378):547-51. doi: 10.1038/nature10648. PMID: 22056989; PMCID: PMC3245796.

Ryan, S. D., Dolatabadi, N., Chan, S. F., Zhang, X., Akhtar, M. W., Parker, J., ... Lipton, S. A.(2013). Isogenic human iPSC Parkinson's model shows nitrosative stress-induced dysfunction in MEF2-PGC1alpha transcription. *Cell*, 155, 1351–1364.

Weykopf B, Haupt S, Jungverdorben J, Flitsch LJ, Hebisch M, Liu GH, Suzuki K, Belmonte JCI, Peitz M, Blaess S, Till A, Brüstle O. Induced pluripotent stem cell-based modeling of mutant LRRK2-associated Parkinson's disease. Eur J Neurosci. 2019 Feb;49(4):561-589. doi: 10.1111/ejn.14345. PMID: 30656775; PMCID: PMC7114274.

Fonseca-Ornelas L, Stricker JMS, Soriano-Cruz S, Weykopf B, Dettmer U, Muratore CR, Scherzer CR, Selkoe DJ. Parkinson-causing mutations in LRRK2 impair the physiological tetramerization of endogenous α-synuclein in human neurons. NPJ Parkinsons Dis. 2022 Sep 16;8(1):118. doi: 10.1038/s41531-022-00380-1. PMID: 36114228; PMCID: PMC9481630.