Jul 24, 2025

Generation of Dopaminergic (DA) neurons from human ES cells

DOI
https://dx.doi.org/10.17504/protocols.io.q26g7nbd1lwz/v1

Arun Thiruvalluvan[1,2], Agnete Kirkeby[1,2]

[1]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA.; [2]Novo Nordisk Foundation Center for Stem Cell Medicine (renew), University of Copenhagen, 2200 Copenhagen, Denmark

Laura Castilla-Vallmanya
Lund University

DOI: https://dx.doi.org/10.17504/protocols.io.q26g7nbd1lwz/v1

Protocol Citation: Arun Thiruvalluvan, Agnete Kirkeby 2025. Generation of Dopaminergic (DA) neurons from human ES cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7nbd1lwz/v1

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

Created: July 16, 2025

Last Modified: July 24, 2025

Protocol Integer ID: 222631

Keywords: ASAPCRN, human ventral midbrain dopaminergic progenitor, generation of dopaminergic, mature dopaminergic neuron, neurons from human es cell, human es cell, dopaminergic, neuron, generation

Funders Acknowledgements:
Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research
Grant ID: ASAP-000520
Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research
Grant ID: ASAP-024296
Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research
Grant ID: ASAP-025170

Abstract

This protocol details how to generate mature dopaminergic neurons from human ventral midbrain dopaminergic progenitors.

Generation of high-purity human ventral midbrain dopaminergic progenitors

1 Embryonic stem cells were maintained and differentiated towards ventral midbrain (vMB) fate based on a
previously published protocol (**dx.doi.org/10.17504/protocols.io.kxygx4eqol8j/v1**)

Terminal differentiation to DA neurons

2 Post differentiation towards ventral midbrain specification, day 16 progenitors were plated for terminal differentiation on Lam-111 (i.e., 2 µg/cm2; Biolaminin: LN111-0501) plates in Neurobasal medium containing:

	Reagent	Dilution/Concentration	Provider	Cat# Number
	NB-21 supplement without vitamin A	1:500	Miltenyi Biotec	130-093-566
	Penicillin/streptomycin	1:1000	Thermo Fisher Scientific	15140122
	L-glutamine	1:1000	Thermo Fisher Scientific	25030081
	NEAA	1:1000	Thermo Fisher Scientific	11140050
	BDNF	20 ng/ml	Miltenyi Biotec	130-096-286
	Ascorbic acid	0.2 mM	Sigma-Aldrich	A4403-100MG
	GDNF	10 ng/ml	Miltenyi Biotec	130-129-548
	db-cAMP	500 µM	Sigma-Aldrich	D0627-1G

Media composition for terminal differentiation of DA neurons

3 Day 16 ventral midbrain progenitors were plated onto Lam-111-coated plates (2 µg/cm^2) at a density of 155,000 cells/cm^2.

4 Every 2–3 days, remove old medium and add new medium to the cells: B27 medium + BDNF (20 ng/ml) + AA (0.2 mM) + GDNF (10 ng/ml) + db-cAMP (500 µM) + DAPT (1 µM). Add 300–500 µl of medium per cm^2, depending on cell confluency.

5 Mature DA neurons phenotypes start to appear from around day 42 and onward, and the neurons can be characterised by immunocytochemistry for mature DA markers such as TH, LMX1, EN1 and MAP2.