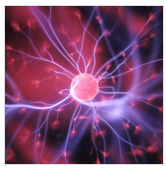

Sep 16, 2020

iPSC Differentiation

In 1 collection

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1 *Works for me* This protocol is published without a DOI.

Neurodegeneration Method Development Community
Tech. support email: **ndcn-help@chanzuckerberg.com**

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protocols.io

ABSTRACT

This protocol explains the iPSC differentiation of lines ND1014, N1, and ND27760 from Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons.

EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6344911/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Xue Y, Zhan X, Sun S, et al. Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons. Stem Cells Transl Med. 2019;8(2):112–123. doi:10.1002/sctm.18-0036

PROTOCOL CITATION

Yingchao Xue, Xiping Zhan, Shisheng Sun, Senthilkumar S. Karuppagounder, Shuli Xia, Valina L Dawson, Ted M Dawson, John Laterra, Jianmin Zhang, Mingyao Ying 2020. iPSC Differentiation. **protocols.io** https://protocols.io/view/ipsc-differentiation-9u7h6zn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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COLLECTIONS

Protocols for Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons

KEYWORDS

ipsc, Atoh1, Ngn2, phosphosite modification, midbrain dopaminergic, differentiation, nonmuscle myosin II, NM-II

LICENSE

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Nov 27, 2019 Liz Brydon Protocols.io

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PARENT PROTOCOLS

Part of collection

Protocols for Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons

MATERIALS

NAME	CATALOG #	VENDOR
mTeSR™1 500 mL Kit	5850	Stemcell Technologies
ACCUTASE™	07920	Stemcell Technologies
MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)	M6494	Thermo Fisher
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix	356231	Corning

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for safety and environmental hazards.

BEFORE STARTING

Obtain approval to work with human stem cells from an appropriate Institutional Review Board.

Day 1-3

1 Plate iPSCs at a density of 1.5×10^5cells per cm^2 in a 12-well plate pre-coated with growth-factor-reduced Matrigel.

Culture medium should be changed daily, and gradually shifted from mTeSR1 to N2 (Thermo Fisher Scientific) in 3 days. The medium also contains [M]**100 Mass Percent SHH** , [M]**100 Mass Percent FGF8b** , and [M]**10 Micromolar (µM) DAPT** .

2 Transfect iPSCs with A-SA mRNA for 3 days, changing media daily.

Day 4

3 Transfect iPSCs with N-SA mRNA for 1 day.

Day 5

4 Dissociate cells using Accutase.

5 Replate with neuron medium in poly-d-lysine/laminin-coated plates at the density of 1×10^5 cells per cm^2.

Neuron medium contains: neurobasal medium with B27 supplement, [M]**10 Mass Percent BDNF** , [M]**10 Mass Percent GDNF** , [M]**1 Mass Percent TGFβ-3** , [M]**0.1 Milimolar (mM) cAMP** , [M]**0.2 Milimolar (mM) ascorbic acid** , and [M]**10 Micromolar (μM) DAPT** .

6

Cells can also be cryopreserved in medium containing 40% neurobasal medium with B27 supplement, 50% fetal bovine serum and 10% DMSO.

Neuron Culture

7 Change media after 48 hours to remove unattached cells followed by half change every 3–4 days during in vitro maturation.

8 Detect cell proliferation and death analysis using MTT and LDH kits, respectively.