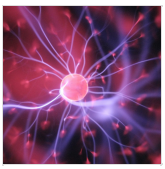

Sep 16, 2020

Immunofluorescence and Cell Counting

In 1 collection

Yingchao Xue[1,2], Xiping Zhan[3], Shisheng Sun[4], Senthilkumar S. Karuppagounder[5,6,7], Shuli Xia[2,5], Valina L Dawson[5,6,7,8,9], Ted M Dawson[5,6,7,8,10], John Laterra[2,5,8,11], Jianmin Zhang[1], Mingyao Ying[2,5]

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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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ABSTRACT

This protocol explains Immunofluorescence and Cell Counting for 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

Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons. Xue Y, Zhan X, Sun S, Karuppagounder SS, Xia S, Dawson VL, Dawson TM, Laterra J, Zhang J, Ying M. Stem Cells Transl Med. 2019 Feb;8(2):112-123. doi: 10.1002/sctm.18-0036. Epub 2018 Nov 1. PMID: 30387318

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. Immunofluorescence and Cell Counting. **protocols.io**
https://protocols.io/view/immunofluorescence-and-cell-counting-9vah62e

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

Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons. Xue Y, Zhan X, Sun S, Karuppagounder SS, Xia S, Dawson VL, Dawson TM, Laterra J, Zhang J, Ying M. Stem Cells Transl Med. 2019 Feb;8(2):112-123. doi: 10.1002/sctm.18-0036. Epub 2018 Nov 1. PMID: 30387318

EXTERNAL LINK

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

COLLECTIONS

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

KEYWORDS

ND1014, N1, ND27760, ipsc, SNCA, Atoh2, Ngn2, immunofluorescence

CREATED

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LAST MODIFIED

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OWNERSHIP HISTORY

Nov 27, 2019 Liz Brydon Protocols.io

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PROTOCOL INTEGER ID

30338

PARENT PROTOCOLS

Part of collection

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

GUIDELINES

Antibodies for immunofluorescence staining

Antibody	Species	Company	Catalog #	Dilution factor
MONOCLONAL ANTI-FLAG(R) M2-HRP	Mouse	Sigma-Aldrich	A8592-.2MG	1000
LMX1A	Rabbit	millipore	AB10533	100
FOXA2	Rabbit	CST	8186P	200
FOXA2	Mouse	R&D Systems	AF2400-SP	100
DAT	Rat	millipore	MAB369	200
TH	Rabbit	CST	2792S	200
Nurr1	Rabbit	millipore	PA14519	200
β3-Tubulin (Tuj1)	Mouse	Covance	MMS-435P	1000
β3-Tubulin (Tuj1)	Rabbit	CST	5568	1000
Synapsin	Rabbit	CST	2312	200
GIRK2	Rabbit	Abcam	ab66502	200
Myosin IIa	Rabbit	CST	3403S	1000
Myosin IIb	Rabbit	CST	3404S	1000
Neurogenin 2	Rabbit	CST	13144S	1000

MATERIALS

NAME	CATALOG #	VENDOR
Triton(R) X-100 100ml	H5142	Promega
4% Paraformaldehyde in PBS	J61899-AK	Alfa Aesar
Goat Serum	16210-064	Gibco - Thermo Fischer
DAPI	62248	Thermo Fisher Scientific
ProLong™ Gold Antifade Mountant	P36930	Thermo Fisher

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.

Immunofluorescence

1 Fix cells in 4% paraformaldehyde in PBS pH **7.4** .

2 Block cells in 5% normal goat serum and 0.2% Triton X-100.

3 Dilute primary antibodies in 5% normal goat serum. Primary antibodies are listed in the "Guidelines".

4 

Incubate samples with primary antibodies **Overnight** at **4 °C** .

5 Stain using Cy3- and Alexa Fluor 488-labeled secondary antibodies.

6 Counterstain with DAPI.

7 

Mount on glass slides using ProLong antifade.

Cell Counting

8 Randomly select 10 different fields to use for counting the number of DAPI-positive cells expressing specific markers in ImageJ. Perform this with at least three independent experiments.