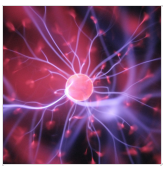

Sep 16, 2020

Western Blot

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 Western Blot 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. Western Blot. **protocols.io** https://protocols.io/view/western-blot-9u9h6z6

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, western blot

CREATED

Nov 27, 2019

LAST MODIFIED

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OWNERSHIP HISTORY

Nov 27, 2019 Liz Brydon Protocols.io

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PROTOCOL INTEGER ID

30337

PARENT PROTOCOLS

In steps of
Example

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
RIPA Buffer	R0278	Sigma Aldrich
Protease Inhibitor Cocktail Set X EDTA-Free	539196	Emdmillipore
Phosphatase Inhibitor Cocktail Set I	524624	Emdmillipore

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.

Protein Extraction

1 Extract total cellular proteins using RIPA buffer containing protease and phosphatase inhibitors.

Gel Electrophoresis

2 Perform SDS-polyacrylamide gel electrophoresis with 50mg total cellular proteins per lane using a 4%–12% gradient Tris-glycine gel.

Western Blot

3 Perform western blot using the Quantitative Western Blot System (LI-COR Biosciences). Primary antibodies are listed in the "Guidelines". Use secondary antibodies labeled with IR dyes.

4 

Quantify protein levels using Odyssey IR Imaging System (LI-COR Biosciences).