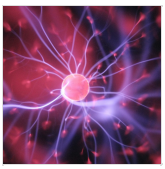

Sep 16, 2020

Electrophysiological Recordings

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 Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) 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

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. Electrophysiological Recordings. **protocols.io** https://protocols.io/view/electrophysiological-recordings-9vbh62n

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

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COLLECTIONS

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

KEYWORDS

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

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PARENT PROTOCOLS

Part of collection

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

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.

Voltage-Clamp Recording

1 Perform voltage-clamp recordings at **35 °C** in a chamber perfused with regular artificial cerebrospinal fluid flowing at 3 ml/minute.

Regular artificial cerebrospinal fluid:

- [M]**124 Milimolar (mM) NaCl**
- [M]**2.5 Milimolar (mM) KCl**
- [M]**1.3 Milimolar (mM) MgCl2**
- [M]**2.5 Milimolar (mM) CaCl2**
- [M]**1 Milimolar (mM) NaH2PO4**
- [M]**26.2 Milimolar (mM) NaHCO3**
- [M]**20 Milimolar (mM) glucose** at pH**7.4** , equilibrated with 95% O_2 and 5% CO_2, ~310 mosm)

Patch electrodes should be pulled from borosilicate glass and have resistances of 2.0–4.0 MΩ when filled with an intracellular solution.

Intracellular solution:

- [M]**135 Milimolar (mM) KMeSO4**
- [M]**5 Milimolar (mM) KCl**
- [M]**5 Milimolar (mM) HEPES** ,

- [M]0.25 Milimolar (mM) EGTA-free acid
- [M]2 Milimolar (mM) Mg-ATP
- [M]0.5 Milimolar (mM) GTP
- [M]10 Milimolar (mM) phosphocreatine-tris at pH7.3 , ~290 mosm

2

Identify neurons using a ×10 objective mounted on an upright microscope with transmitted light.

3 Visualize their neuronal somata through a ×40 water immersion objective using IR differential interference contrast optics.

4 Cell somatic recordings should be made using an Axopatch 200B amplifier in combination with pClamp 10.7 software (Molecular Devices).

- Voltage clamp at −70 mV.
- Monitor R_{series} and R_{input} using a 2.5-mV 100-ms depolarizing voltage step in each recording sweep.
- Filter current traces at 5 kHz, digitize at 10 kHz using a Digidata 1440 interface, and store for off-line analysis.
- Monitor resting membrane potential and the action potential for more than 5 minutes before drug applications.

5 Add Tetraethylammonium (TEA) and ZD 7288 (Sigma-Aldrich) the artificial cerebrospinal fluid, to block K+ or I_h currents, respectively.