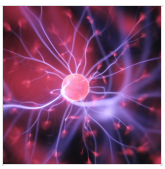

Sep 16, 2020

6-Hydroxydopamine (6-OHDA) Treatment and Neurite Tracing in miDA Neurons

In 1 collection

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

Neurodegeneration Method Development Community
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ABSTRACT

This protocol describes 6-Hydroxydopamine (6-OHDA) Treatment and Neurite Tracing in miDA Neurons 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. 6-Hydroxydopamine (6-OHDA) Treatment and Neurite Tracing in miDA Neurons. **protocols.io** https://protocols.io/view/6-hydroxydopamine-6-ohda-treatment-and-neurite-tra-9zfh73n

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

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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, 6-Hydroxydopamine (6-OHDA), Neurite Tracing, miDA, Neurons

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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
Calcein, AM, cell-permeant dye	C3100MP	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.

Cell Seeding

1 Plate miDA neurons (3×10^3 per well) in a 384-well plate coated with poly-D-lysine and laminin.

Calcein AM Assay

2 Prepare fresh 6-OHDA in vehicle solution (0.15% ascorbic acid in H_2O).

Control cells should be treated with vehicle solution alone.

3

Incubate cells with fresh 6-OHDA solution for **24:00:00** .

4 Stain live cells with [M]**1 Micromolar (µM) calcein-AM** .

The calcein AM assay is based on the conversion of the cell permeant nonfluorescent calcein AM dye to the fluorescent calcein dye by intracellular esterase activity in live cells.

5

Image using a confocal microscope.

Calcein-AM Analysis

6

Quantify neurite length using high content analysis software (HCA-Vision V2.2.0. CSIRO).