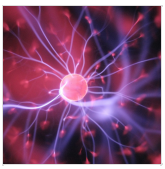

Sep 16, 2020

High-Performance Liquid Chromatography (HPLC)

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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ABSTRACT

This protocol describes High-Performance Liquid Chromatography (HPLC) 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. High-Performance Liquid Chromatography (HPLC). **protocols.io** https://protocols.io/view/high-performance-liquid-chromatography-hplc-9zdh726

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, HPLC

CREATED

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OWNERSHIP HISTORY

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PROTOCOL INTEGER ID

30469

PARENT PROTOCOLS

Part of collection

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

MATERIALS

NAME	CATALOG #	VENDOR
Potassium chloride	P9333	Sigma Aldrich
Perchloric acid	244252	Sigma – Aldrich
Savant SPD121P SpeedVac Kit	SPD121P	Thermo Scientific
Savant Refrigerated Vapor Traps	RVT5105	Thermo Scientific

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.

HPLC Preparation

1 Replace neuron culture medium with Hanks' balanced saline solution buffer with the addition of **[M]56 Milimolar (mM) KCl** .

2

Incubate for **00:15:00** at **37 °C** .

3

Collect media and centrifuge to clear cell debris. Collect neuron pellet.

4

Freeze immediately and store at **-80 °C** .

HPLC Analysis

5 Thaw samples.

6 Concentrate using a vacuum (Savant SPD 121P) connected with a refrigerated vapor trap (Savant RVT 5105).

7 Resuspend freeze-dried samples in **10 Milimolar (mM) perchloric acid** .

8 

Analyze monoamines using HPLC electrochemical detection by dual channel Coulochem III electrochemical detector (model 5300).

9 Separate monoamines using a reverse phase C18 column (3-mm 3 150-mm C-18 RP-column) with a flow rate of 0.600 ml/minute.

10 

Quantify monoamine concentrations by comparison of the area under the curve to known standard dilutions.