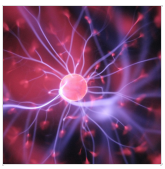

Sep 16, 2020

Immunoprecipitation (IP) and Mass Spectrometry

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 explains Immunoprecipitation (IP) and Mass Spectrometry 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. Immunoprecipitation (IP) and Mass Spectrometry. **protocols.io**
https://protocols.io/view/immunoprecipitation-ip-and-mass-spectrometry-9zhh736

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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, Immunoprecipitation, IP, Mass Spectrometry, MS

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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
Iodoacetamide	AK-U470	P212121
Sodium bicarbonate	S6014	Sigma Aldrich
Sequencing Grade Modified Trypsin, 100ug	V5117	Promega
TCEP-HCl	TCEP	Gold Biotechnology
Urea	U5378	Sigma Aldrich
NE-PER Nuclear and Cytoplasmic Extraction Reagents	78833	Thermo Scientific
Monoclonal ANTI-FLAG M2 antibody produced in mouse	F3165	Sigma Aldrich
Dynabeads Protein G for Immunoprecipitation	10003D	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.

Immunoprecipitation (IP)

1 Twenty-four hours after mRNA transfection, extract nuclear proteins of iPSCs using the NE-PER Nuclear and Cytoplasmic Extraction Kit.

2 Perform FLAG IP using anti-FLAG M2 antibody and Protein G Dynabeads following the manufacturer's protocol.

3 Elute binding proteins by elution buffer ([M]**8 Molarity (M) urea** , [M]**1 Molarity (M) NaHCO3 in water**).

Mass Spectrometry (MS)

4 Denature protein samples in [M]**8 Molarity (M) urea** / [M]**1 Molarity (M) NH4HCO3 buffer** .

5 

Reduce protein samples by [M]**10 Milimolar (mM) TCEP** at **37 °C** for **01:00:00** .

6 

Alkylate protein samples by [M]**15 Milimolar (mM) iodoacetamide** at **Room temperature** in the dark for **00:30:00** .

7 Dilute samples fivefold with deionized water.

8 Add sequencing grade trypsin (50:1, w/w) to the samples.

9 

Incubate at **37 °C** **Overnight** with shaking.

10 

Centrifuge at **13000 x g, 00:10:00** to remove any particulate matter.

11 Purify by a C18 solid-phase extraction.

12 Elute peptides from the C18 column in 60% ACN/0.1% TFA.

13 Measure peptide concentrations using a NanoDrop microvolume spectrophotometer.

14 Resuspend in 0.2% FA solution.

15 Inject 1 µg in 6 µl solution for each LC–MS/MS analysis using Velos Orbitrap mass spectrometer with a 120-minute

online LC separation.

IP and MS Analysis

16 Search raw data against UniProt human protein database by Proteome Discoverer with the following parameters:
- two missed cleavages allowed for trypsin digestion
- carbamidomethyl (C) set as a fixed modification
- oxidation (M) and acetyl (protein N-terminal) set as variable modifications

17 

Normalize the intensity of each identified proteins to the intensity of their A-WT or A-SA proteins, respectively, and further calculate the A-SA/A-WT ratio of normalized intensity.