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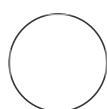
## 🌐 iNeuron differentiation from human iPSCs

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### ABSTRACT

We adapted a previously-described method (Boecker et al., 2020, 2021; Fernandopulle et al., 2018) for differentiating iPSCs stably expressing mNGN2 at a safe-harbor locus into human excitatory glutamatergic neurons. Pre-i 3Neuron iPSCs (human iPSCs with an integrated doxycycline-inducible mNGN2 transgene in the AAVS1 safe-harbor locus) were a gift from M. Ward (National Institutes of Health, Maryland).

### ATTACHMENTS

[548-1144.pdf](#)

### GUIDELINES

#### Citations:

- Boecker, C.A., and Holzbaur, E.L.F. (2021). Hyperactive LRRK2 kinase impairs the trafficking of axonal autophagosomes. *Autophagy* 00, 1–3.
- Boecker, C.A., Olenick, M.A., Gallagher, E.R., Ward, M.E., and Holzbaur, E.L.F. (2020). ToolBox: Live Imaging of intracellular organelle transport in induced pluripotent stem cell-derived neurons. *Traffic* 21, 138–155.
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**Protocol status:** Working  
We use this protocol and it's working

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**Keywords:** iPSC, Differentiation, iNeuron, NGN2, i3 Neuron

## MATERIALS

### Materials

- 10 cm cell culture dish
- 15 cm cell culture dish
- Cryovials

### Reagents

-  Growth Factor Reduced (GFR) Matrigel® Corning Catalog #354230
-  Essential 8™ Medium Gibco, ThermoFisher Catalog #A1517001
-  ACCUTASE™ STEMCELL Technologies Inc. Catalog #07920
-  DMEM/F-12, HEPES Thermo Fisher Scientific Catalog #11330032
-  N-2 Supplement (100X) Thermo Fisher Catalog #17502048
-  MEM Non-Essential Amino Acids Solution (100X) Thermo Fisher Catalog #11140050
-  GlutaMAX™ Supplement Thermo Fisher Catalog #35050061
-  Doxycycline hydrochloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #D9891
-  Y-27632 Selleckchem Catalog #S1049
- 
-  Fetal bovine serum for cell culture (tetracycline-free) Takara Bio Inc. Catalog #631107
- DMSO (CATALOG)
-  BrainPhys™ Neuronal Medium 500 mL STEMCELL Technologies Inc. Catalog #5790
-  Animal-Free Recombinant Human NT-3 peprotech Catalog #AF-450-03
-  Recombinant Human/Murine/Rat BDNF peprotech Catalog #450-02
-  B-27 Supplement Gibco - Thermo Fischer Catalog #17504044

## SAFETY WARNINGS



Wear proper PPE when transferring cryovials to liquid N<sub>2</sub>.


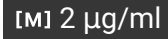

## iNeuron differentiation from human iPSCs

1w


- 1 Culture pre-iNeuron iPSCs in a 10 cm dish coated with Growth Factor Reduced Matrigel in Essential 8 media, fed daily.


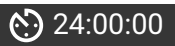

### Note

Pre-iNeuron iPSCs should either have doxycycline-inducible NGN2 present in the safe-harbor AAVS1 locus (“i3Neurons”) or should stably express doxycycline-inducible NGN2 following piggybac transfection (see protocol: “Piggybac-mediated stable expression of NGN2 in iPSCs for differentiation into excitatory glutamatergic neurons”). Before performing differentiation, iPSCs should be tested for mycoplasma, and cytogenetic analysis of G-banded metaphase cells should be performed to confirm a normal karyotype.

- 2  Passage iPSCs using warm Accutase and plate  $5.5 \times 10^6$  cells onto a Matrigelcoated 15 cm dish, in Induction Media (DMEM/F12 supplemented with 1% N2- supplement [GIBCO], 1% NEAA [GIBCO], and 1% GlutaMAX [GIBCO], and containing  2 µg/ml doxycycline and  10 µm ROCK inhibitor).

### Note

DMEM/F12 supplemented with N2-supplement, NEAA and GlutaMAX can be kept at  4 °C for 2-3 months. Doxycycline and ROCK inhibitor should always be added fresh.

- 3  After  24:00:00 , replace all media with fresh Induction Media, containing  2 µg/ml doxycycline but no ROCK inhibitor.

1d

- 3.1  Replace again with the same media after  24:00:00 (  48:00:00 after plating).

3d

## 4 72 hours after plating, dissociate cells with warm Accutase.

### 4.1 Count cells in freezing media.



#### Freezing media

A	B
BrainPhys	70%
FBS	20%
DMSO	10%
BDNF	10 ng/mL
NT-3	10 ng/mL
B-27 supplement	1x

### 4.2 Freeze down cells in a Mr. Frosty container placed in a -80 °C freezer Overnight .



### 4.3 On the following day, transfer cryopreserved neurons to liquid nitrogen storage.

