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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 N2.

## 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 x $10^6$ 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 [M] 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 [M] 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.