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iNeuron differentiation from human iPSCs

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We use this protocol and it's working

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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

PDF

548-1144.pdf

149KB

Guidelines

Citations:

- Boecker, C.A., and Holzbaur, E.L.F. (2021). Hyperactive LRRK2 kinase impairs the trafficking of axonal autophagosomes. Autophagy 00, 1–3.
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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 - Thermo Fisher Scientific** **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 Fisher Scientific** **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 × 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.