Feb 23, 2026

hiPSC-iAstro SOX9 NFIB Induction

DOI
https://dx.doi.org/10.17504/protocols.io.x54v9bodml3e/v1

Nadine Schrode[1], Kristen Brennand[1]

[1]Icahn School of Medicine at Mount Sinai

Brennand Laboratory

Kristen Brennand
Yale University School of Medicine

DOI: https://dx.doi.org/10.17504/protocols.io.x54v9bodml3e/v1

Protocol Citation: Nadine Schrode, Kristen Brennand 2026. hiPSC-iAstro SOX9 NFIB Induction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9bodml3e/v1

Protocol status: Working
We use this protocol and it's working

Created: February 20, 2026

Last Modified: February 23, 2026

Protocol Integer ID: 243770

Keywords: hiPSCs, NPCs, neuron, MPRA, CRISPR, FACS, morphology, iastro sox9 nfib induction direct induction of astrocyte, iastro sox9 nfib induction, astrocyte, hipsc

Funders Acknowledgements:
Kristen Brennand
Grant ID: R56MH101454

Abstract

Direct induction of astrocytes from hiPSCs using transcription factor induction

Guidelines

1. Doxycycline: 25 mg/mL dissolved in water (10000x stock). Sterilize with a 0.22 µm filter and store at -20°C. Protect from light.
2. CNTF: Reconstitute at 10 µg/ml in sterile 10 mM sodium phosphate containing 0.1% BSA. Aliquot and store at -20°C.
3. bFGF: Reconstitute at 1 mg/ml in sterile 5mM Tris, pH 7.6 containing 0.1% BSA. Aliquot and store at -20°C.
4. BMP4: Reconstitute at 10 µg/ml in sterile 4 mM HCl containing 0.1% BSA. Aliquot and store at -20°C.
5. dbcAMP: Reconstitute at 100 mg/ml in sterile water. Aliquot and store at -20°C. Protect from light.
6. N-acetyl-cysteine: Reconstitute at 50 mg/ml in sterile water. Aliquot and store at -20°C.
7. Heparin-binding EGF-like growth factor: Reconstitute at 50 µg/ml in sterile PBS containing 0.1% BSA. Aliquot and store at -20°C.

Materials

- DMEM/F-12 (ThermoFisher Scientific, cat. no. 31330038)
- Neurobasal (ThermoFisher Scientific, cat. no. 21103049)
- FBS (ThermoFisher Scientific, cat. no. 10082147)
- N2 supplement (ThermoFisher Scientific, cat. no. 17502001)
- B27 supplement (ThermoFisher Scientific, cat. no. 17504001)
- NEAA (ThermoFisher Scientific, cat. no. 11140035)
- Glutamax (ThermoFisher Scientific, cat. no. 35050061)
- Sodium Pyruvate (ThermoFisher Scientific, cat. no. 11360070)
- bFGF (Peprotech, cat. no. 100-18B)
- CNTF (Peprotech, cat. no. 450-13)
- BMP4 (Peprotech, cat. no. 120-05ET)
- dbcAMP (Sigma-Aldrich, cat. no. D0627-25MG)
- N-acetyl-cysteine (Sigma-Aldrich, cat. no. A8199-10G)
- Heparin-binding EGF-like growth factor (Sigma-Aldrich, cat. no. E4643-50UG)
- Polybrene (Sigma-Aldrich, cat. no. TR-1003-G)
- Doxycycline (Sigma-Aldrich, cat. no. D9891-100UG)
- Accutase (ThermoFisher Scientific, cat. no. A1110501)
- Matrigel (Corning, cat. no. 354230)
- Rock inhibitor (StemCell Technologies, cat. no. Y-27632)

Procedure

1 Day -2: Reagent setup

2 Expansion Medium:
DMEM/F-12
10% FBS
1% N2 supplement 1% Glutamax

3 FGF Medium:
Neurobasal
2% B27 supplement 1% NEAA
1% Glutamax
1% FBS
8 ng/ml FGF
5 ng/ml CNTF 10 ng/ml BMP4

4 Maturation Medium:
1:1 DMEM/F-12 and Neurobasal
1% N2
1% Sodium Pyruvate
1% Glutamax
5 µg/ml N-acetyl-cysteine
5 ng/ml heparin-binding EGF-like growth factor 10 ng/ml CNTF
10 ng/ml BMP4
500 µg/ml dbcAMP

5 Day -1
Dissociate hiPSC with Accutase and incubate 5×10^5^ cells with virus for 1 h.

6 Seed in Matrigel-coated 6-well plates with *StemFlex* containing 10 µM Rock inhibitor

7 Spinfect with Sox9 and NfiB virus for 1h at 1000g

8 Day 0
Aspirate medium and add 2 ml of fresh *StemFlex* containing 2.5 µg/ml of Doxycycline per well.

9 Days 1 and 2

Aspirate medium and add 2 ml of *Expansion Medium* containing 2.5 µg/ml of Doxycycline and pertinent antibiotics (for whatever viruses you used).

10 Day 3
Aspirate medium and add 2 ml of 3/4 of *Expansion Medium* and 1/4 of *FGF Medium* containing 2.5 µg/ml of Doxycycline per well.

11 Day 4
Aspirate medium and add 2 ml of 1/2 of *Expansion Medium* and *FGF Medium* containing 2.5 µg/ml of Doxycycline per well.

12 Day 5
Aspirate medium and add 2 ml of 1/4 of *Expansion Medium* and 3/4 of *FGF Medium* containing 2.5 µg/ml of Doxycycline per well.

13 Day 6
Aspirate medium and add 2 ml of *FGF Medium* containing 2.5 µg/ml of Doxycycline.

14 Day 7
Dissociate cells with Accutase and pellet at 400g for 5 min.

15 Replate cells in Matrigel-coated coverslips, petri dishes or wells according to desired output.

16 Day 8
Use the necessary amount of *FGF Medium* containing 2.5 µg/ml of Doxycycline.

17 Aspirate medium and add 1/4 *FGF Medium* and 3/4 *Maturation Medium* containing 2.5 µg/ml of Doxycycline.

18 Day 10
Aspirate *half* of the medium and add the same amount of *Maturation Medium* containing 2.5 µg/ml of Doxycycline.

19 From here, change *half* of the *medium* every 2-3 days.