ASTROCYTE PRODUCTION (Support Protocol 7.1)

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EXTERNSL LINK
https://doi.org/10.1002/cpcb.51

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

doi: https://doi.org/10.1002/cpcb.51

ATTACHMENTS
fernandopulle2018.pdf

DOI
dx.doi.org/10.17504/protocols.io.5xag7ie

EXTERNSL LINK
https://doi.org/10.1002/cpcb.51

PROTOCOL CITATION
Michael S. Fernandopulle, Ryan Prestil, Christopher Grunseich, Chao Wang, Li Gan, Michael E. Ward 2019. ASTROCYTE PRODUCTION (Support Protocol 7.1). protocols.io
https://dx.doi.org/10.17504/protocols.io.5xag7ie

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

doi: https://doi.org/10.1002/cpcb.51

COLLECTIONS

Transcription Factor-Mediated Differentiation of Human iPSCs into Neurons

KEYWORDS
i3LMN, i3Neurons, iPSC, iPSC-derived neurons, transcription factor-mediated differentiation

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Citation: Michael S. Fernandopulle, Ryan Prestil, Christopher Grunseich, Chao Wang, Li Gan, Michael E. Ward (12/18/2019). ASTROCYTE PRODUCTION (Support Protocol 7.1). https://dx.doi.org/10.17504/protocols.io.5xag7ie

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MATERIALS TEXT

- P0 or P1 mouse pups
- DMEM, high glucose (Gibco, cat. no. 11965092) containing 10% (v/v) DMEM, high glucose
  - Thermo Fisher Scientific Catalog #11965092
- FBS (Gibco, cat. no. 16140071)
  - Fetal Bovine Serum, qualified, heat inactivated, United States Thermo Fisher Scientific Catalog #16140071
- Trypsin (Gibco, cat. no. 25300054)
  - Trypsin-EDTA (0.05%), phenol red Thermo Fisher Scientific Catalog #25300054
- Shaker
- 75-cm² (T75) culture flasks (Thermo, cat. no. 156499)

Additional reagents and equipment for cell culture (see Basic Protocol 1) and counting cells (Phelan & May, 2015)

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

1 Use 3 P0 or 1 P1 rat pup per uncoated T75 flask. Meninges should be completely removed from brains, and astrocytes...
isolated per standard mechanical and/or enzymatic dissociation protocols under sterile conditions. Expand astrocytes for 1 week or until confluent in DMEM containing 10% FBS by volume (astrocyte medium).

2. Shake at 300 rpm at \(37 \degree C\) to remove microglia, any surviving neurons, and other contaminating cell types.

3. When the flask nears confluency, wash with PBS and incubate with trypsin for 00:05:00.

4. Centrifuge 00:05:00 at 200 \(x\)  \(g\), Room temperature.

5. Aspirate supernatant.

6. Resuspend in DMEM containing 10% FBS.

7. Seed cells from each T75 into three new T75 flasks and grow until nearly confluent.

   Astrocytes may be passaged in culture up to two times. Passaging more than two times reduces neutrophic and synaptogenic support qualities.

8. Alternatively, astrocytes can be frozen in liquid nitrogen (see Basic Protocol 1).

9. To add astrocytes to neural cultures, repeat steps 3 to 6 or thaw from frozen stock, and seed cells onto a transwell.

   Over-adding astrocytes is better than under-adding; if insufficient number of astrocytes are plated, neurons will likely be less healthy. The induction can largely be rescued by adding additional astrocytes the following day if it appears that the neurons are not responding well.