iPSCs

Dec 18, 2019

ASTROCYTE PRODUCTION (Support Protocol 7.1)

In 1 collection

Michael S. Fernandopulle[1], Ryan Prestil[1], Christopher Grunseich[1], Chao Wang[2], Li Gan[2], Michael E. Ward[1]

[1]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, [2]Gladstone Institute of Neurological Disease, Gladstone Institutes, San Francisco, California

1 Works for me dx.doi.org/10.17504/protocols.io.5xag7ie

Neurodegeneration Method Development Community

EXTERNAL LINK

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Fernandopulle, M. S., Prestil, R., Grunseich, C., Wang, C., Gan, L., & Ward, M. E. (2018). Transcription-factor mediated differentiation of human iPSCs into neurons. Current Protocols in Cell Biology, e51. doi:https://doi.org/10.1002/cpcb.51

fernandopulle2018.pdf

MATERIALS TEXT

- P0 or P1 mouse pups
- DMEM, high glucose (Gibco, cat. no. 11965092) containing 10% (v/v)

DMEM, high glucose
by Thermo Fisher Scientific
Catalog #: 11965092

- FBS (Gibco, cat. no. 16140071)

Fetal Bovine Serum, qualified, heat inactivated, United States
by Thermo Fisher Scientific
Catalog #: 16140071

- Trypsin (Gibco, cat. no. 25300054)

Trypsin-EDTA (0.05%), phenol red
by Thermo Fisher Scientific
Catalog #: 25300054

- Shaker
- 75-cm^2 (T75) culture flasks (Thermo, cat. no. 156499)

Nunc™ EasYFlask™ Cell Culture Flasks
Cell Culture Flasks
Thermo Scientific™ 156499

- Transwells (e.g., for 12-well plates; Corning, cat. no. 3401)

12 mm Transwell® with 0.4 µm Pore Polycarbonate Membrane Insert, Sterile
Corning 3401

- 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 °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.