




Version 2 ▼

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Astrocyte extraction from brain organoids

V.2

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ABSTRACT

Protocol for astrocyte extraction from brain organoids.

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MATERIALS TEXT

Mature Astrocyte media



Advanced DMEM/F12 (Thermo, 12634-010)	250ml
Neurobasal (Life Tech, 21103049)	250 ml
1% NEAA (Thermo,11140-050)	5 ml
1% Glutamax (Thermo, 35050- 061)	5ml
1% N2 (Life Tech, 17502048)	5 ml
2% B27 (Thermo, 17504044)	10 ml
10ng/ml CNTF (ProSpec, CYT- 272)	250ul

- 1 Coat a 6 well plate coat with gelatin 0.1% or
[Geltrex LDEV Free hESC Quality 5 ml Thermo Fisher Scientific Catalog #A1413302](#)
 1:100


- 2 Collect 10-20 spheres and place in a 6 well plate (day 40+ spheres)

- 3 Wash in PBS twice

- 4 Aspirate the PBS

- 5 Add  1 mL of 5m
[TrypLE™ Select Enzyme \(1X\), no phenol red Thermo Fisher Catalog #12563011](#) for
 00:05:00

- 6 Triturate using glass a pipette (2-3 up and down)

- 7 Transfer the cells and tissue (even the chunks, the trituration is more effective if you have chunks of tissue) to the coated 6 well plate
- 8 Aspirate the extra TrypLe
- 9 Add  3 mL of astrocyte media (<https://www.sciencellonline.com/astrocyte-medium.html>)
- 10 Half media change media every 3 days or until it turns orange. 3d
- 11 Keep changing media every 3d until you observe good amount of cell attached to the plate (may vary according to the patterning)
- 12 Passage the astrocytes to a gelatin or geltrex coated 15 cm plate in Astrocyte media once they reach 80% confluency
- 13 For the experimental protocols after passage cell will be incubated in maturation media (TBD)