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Version 2

Astrocyte extraction from brain organoids V.2

 In 2 collections

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for astrocyte extraction from brain organoids.

Materials

Mature Astrocyte media

	Advanced DMEM/F12 (Thermo, 12634-010)	250 ml
	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)	250 ul

Protocol materials

 Geltrex LDEV Free hESC Quality 5 ml **Thermo Fisher Scientific Catalog #A1413302**

 TrypLE Select Enzyme (1X), no phenol red **Thermo Fisher Catalog #12563011**



- 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)