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Astrocyte extraction from brain organoids V.1

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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.

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 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)