Astrocyte extraction from brain organoids V.1

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Version 1

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1 Works for me

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ABSTRACT

Protocol for astrocyte extraction from brain organoids.

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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 TrypLE™ Select Enzyme (1X), no phenol red **Thermo Fisher Catalog #12563011** for **00:05:00** 5m

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

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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)

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