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Midbrain organoid astrocyte seeding

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Protocol status: Working

We use this protocol and it's working

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Abstract

Midbrain organoid astrocyte seeding protocol

Materials

Midbrain organoid media

D35+ (Long-Term Media) DMEM/F-12+Glutamax + 13 B-27 minus vitamin A + 13 N-2 + 10 ng/mL BDNF + 10 ng/mL GDNF + 0.2 μ M Ascorbic Acid

Protocol materials

 Astrocyte Medium **ScienCell Catalog # #1801**

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



- 1 Refer to the publish protocols to obtain the cell materials required.
<https://www.protocols.io/view/midbrain-organoid-differentiation-in-spinner-flask-rm7vzbnr4vx1/v1>
<https://www.protocols.io/view/astrocyte-extraction-from-brain-organoids-261ge364wl47/v2>

- 2 From a 90% confluent plate of astrocytes.

- 3 Wash with PBS.

- 4 Add 1 mL of TrypLE™ Select Enzyme (1X), no phenol red Thermo Fisher Catalog #12563011.

5m

- 5 Transfer the cells to 15 ml falcon tubes and add 5 ml of media.

- 6  300 rcf, 25°C, 00:03:00

3m

- 7 Resuspend the cell pellet in 1ml of  Astrocyte Medium **ScienCell Catalog # #1801**

- 8 Count the cells using

Equipment

Countess II

NAME

Life Technologies

BRAND

AMQAX1000

SKU

- 9 Individually add day 40 midbrain organoids to a Corning 96 well V bottom shape plate.



- 10 Add 50K astrocyte to the organoids.
- 11 Add 100 ul of organoid media.
- 12 After a week transfer the organoids to a 96 well plate coated with

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

1/100 .