




Oct 27, 2022

Midbrain organoid astrocyte seeding

[gustavo.parfitt](#)¹¹Icahn School of Medicine

1 Works for me

 Sharedx.doi.org/10.17504/protocols.io.rm7vzbyp4vx1/v1**Ahfeldt Lab**

gustavo.parfitt

ABSTRACT

Midbrain organoid astrocyte seeding protocol

DOI

dx.doi.org/10.17504/protocols.io.rm7vzbyp4vx1/v1

PROTOCOL CITATION

gustavo.parfitt 2022. Midbrain organoid astrocyte seeding . **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.rm7vzbyp4vx1/v1>



LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Oct 27, 2022

LAST MODIFIED

Oct 27, 2022


PROTOCOL INTEGER ID


71926

MATERIALS TEXT

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 µM Ascorbic Acid

- 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

 Astrocyte

Resuspend the cell pellet in 1ml of

Medium ScienCell Catalog # #1801
- 8 Count the cells using

Countess II

Life Technologies AMQAX1000
- 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 .