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## 🌐 Midbrain astrocyte and co-culture

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**Protocol status:** Working

**We use this protocol and it's working**

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### Abstract

Midbrain astrocyte and co-culture for mix-genetic experiments.

## Materials

### Media for iPSC

StemFlex medium

### Media for iPSC passaging

StemFlex medium + 10  $\mu$ M Y-27632 + 1:100 Pen/Strep

### Media composition for differentiation

D0-1 - DMEM/F-12+Glutamax + 13 B-27 minus vitamin A + 13 N-2 + 100 nM LDN193189 + 10  $\mu$ M SB431542

D2-3 - DMEM/F-12+Glutamax + 13 B-27 minus vitamin A + 13 N-2 + 100 nM LDN193189 + 10  $\mu$ M SB431542+ 2  $\mu$ M Purmorphamine + 1  $\mu$ M SAG


D4-7 - DMEM/F-12+Glutamax + 13 B-27 minus vitamin A + 13 N-2 + 100 nM LDN193189 + 10  $\mu$ M SB431542 + 2  $\mu$ M Purmorphamine + 1  $\mu$ M SAG + 3  $\mu$ M CHIR99021

D8-11 - DMEM/F-12+Glutamax + 13 B-27 minus vitamin A + 13 N-2 + 100 nM LDN193189 + 3  $\mu$ M CHIR99021

D12-24 (Terminal Media) DMEM/F-12 + Glutamax + 13 B-27 minus vitamin A + 13 N-2 + 20 ng/mL BDNF + 20 ng/mL GDNF + 0.2 mM Ascorbic Acid + 10  $\mu$ M DAPT + 0.1  $\mu$ M dcAMP

D25+ (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  $\mu$ M Ascorbic Acid + 10 ng/mL CNTF.

## Protocol materials

 STEM-CELLBANKER - GMP Grade **amsbio Catalog #11890** Step 20

 Astrocyte Medium **ScienCell Catalog # #1801** Step 29

 ACCUTASE™ 100 mL **STEMCELL Technologies Inc. Catalog #7920** Step 4

 Geltrex LDEV Free hESC Quality 5 ml **Thermo Fisher Scientific Catalog #A1413302** In [2 steps](#)

## Intro

- 1 Midbrain NPC were generated following the publish protocol <https://www.protocols.io/view/midbrain-organoid-differentiation-in-spinner-flask-rm7vzbnr4vx1/v1> with modifications listed below.

## NPC generation

8m

- 2 From a 80% confluent plate of iPSCs in a 10 cm plate.


- 3 Wash with 5ml of PBS

- 4 For the passaging add 3 mL of

 ACCUTASE™ 100 mL **Stemcell Technologies Catalog #7920**

5m

- 5 Add 5 mL of StemFlex , gently mix and transfer to a 15 ml conical tube.

- 6  300 rcf, 25°C, 00:03:00

3m

- 7 Resuspend the cells in 1 mL of StemFlex with rock inhibitor and Count the cells using

### Equipment

**Countess II**

NAME

Life Technologies

BRAND

AMQAX1000

SKU


- 8 Plate 2 million cells in a 1/100

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

coated plate in Stemflex with 10 µM Y-27632.




- 9 D0 change half the media with D0-1 medium.
- 10 D2 change half the media with D2-3 medium.
- 11 D3 Change half the media with D2-3 medium.
- 12 D4 change half the media with D4-7 medium.
- 13 D5 change half the media with D4-7 medium.
- 14 D6 change half the media with D4-7 medium.
- 15 D7 change half the media with D4-7 medium.
- 16 D8 change all the media with D8-11 medium.
- 17 D9 change half the media with D8-11 medium.
- 18 D10 change half the media with D8-11 medium.
- 19 D11 change half the media with D8-11 medium.

20 Freeze the NPCs in  STEM-CELLBANKER - GMP Grade **amsbio Catalog #11890**

## NPC Plating

21 Thaw the NPCs and transfer to a 15 ml conical tube.

22 Add 5 ml of D12 terminal media with 10  $\mu$ M Y-27632

23  300 rcf, 25°C, 00:03:00

3m

24 Resuspend the cells in 1 mL D12 terminal media with 10  $\mu$ M Y-27632 and count the cells using

Equipment

Countess II

NAME

Life Technologies

BRAND

AMQAX1000

SKU

Astrocyte co-culture

3w 4d 0h 3m

25 In a 96 well plate coated with

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

1/100 plate 50k NPCs

26 Change half media every day until day 25.

3w 4d

27 Obtain astrocytes following protocol <https://www.protocols.io/view/astrocyte-extraction-from-brain-organoids-261ge364wl47/v2>

28 Thaw the Astrocytes and transfer to a 15 ml conical tube.

29 Add 5 ml of A  Astrocyte Medium **Sciencell Catalog # #1801** with 10  $\mu$ M Y-27632.

30

 300 rcf, 25°C, 00:03:00

3m

31 Resuspend the cells in 1 mL D12 terminal media with 10  $\mu$ M Y-27632 and count the cells using

Equipment

**Countess II**

NAME

Life Technologies

BRAND

AMQAX1000

SKU

32 Plate 10k on the neurons.

33 Mature the co-cultures until day 50 changing media every other day with day 25 media.