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Midbrain astrocyte and co-culture

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ABSTRACT

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

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MATERIALS TEXT

Media for iPSC
StemFlex medium

Media for iPSC passaging
StemFlex medium + 10 µM 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 µM SB431542

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

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

D8-11 - DMEM/F-12+Glutamax + 13 B-27 minus vitamin A + 13 N-2 + 100 nM LDN193189 + 3 µM 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 µM DAPT + 0.1 µM 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 µM Ascorbic Acid + 10 ng/mL CNTF.

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 bellow.

NPC generation 8m

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

3 Wash with 5ml of PBS

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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

Countess II
Life Technologies AMQAX1000

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.

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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.

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22 Add 5 ml of D12 terminal media with 10 µM Y-27632

23 **300 rcf, 25°C, 00:03:00** 3m

24 Resuspend the cells in 1 mL D12 terminal media with 10 µM Y-27632 and count the cells using

Countess II
Life Technologies AMQAX1000

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 Astrocyte
Add 5 ml of A Medium **ScienCell Catalog # #1801** with 10 µM Y-27632.

30 **300 rcf, 25°C, 00:03:00** 3m

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31 Resuspend the cells in 1 mL D12 terminal media with 10 µM Y-27632 and count the cells using

Countess II

Life Technologies **AMQAX1000**

32 Plate 10k on the neurons.

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

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