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iPSC Midbrain differentiation in 96 well plates

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ASAP Collaborative Rese…

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We use this protocol and it's working

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Abstract

Midbrain differentiation protocol using spinner flasks.

Materials

Media for iPSC
StemFlex medium

Media for the first passage
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

D12-21 (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

D22-34 (Terminal 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 µM DAPT + 0.1 mM dcAMP

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

iPSCs plating for differentiation

8m

1 Passage 1X10^6 iPSC into Geltrex coated 10-cm dish with Accutase and rock inhibitor (refer to material section).

2 For the passaging add 3 mL of Accutase for 00:05:00 . 5m

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

4 300 x g, 25°C, 00:03:00 3m

5 Resuspend the cells in 1 mL of StemFlex with rock inhibitor and Count the cells.

6 Wait until iPSC reaches 60% confluence to start differentiation.

Midbrain Differentiation

1w 5d

7 Starting on day D0, Aspirate all the media, wash the plate with PBS, and add D0-D1 media (refer to the materials).

8 D1 change half the media with D0-1 medium.

9 D2 change half the media with D2-3 medium.

10 D3 Change half the media with D2-3 medium.

11 D4 change half the media with D4-7 medium.

12 D5 change half the media with D4-7 medium.

13 D6 change half the media with D4-7 medium.

14 D7 change half the media with D4-7 medium.

15 D8 change all the media with D8-11 medium.

16 D9 change half the media with D8-11 medium.

17 D10 change half the media with D8-11 medium.

18 On the D12 passage, plate 10000 neural progenitors cells into a Geltrex-coated 96-well plate.

19 Wash the cells with PBS.

20 For the passaging add 5 mL of Accutase for 00:05:00 . 5m

21 Add 5 mL of Day 12 media with rock inhibitor, gently mix and transfer to a 15 ml conical tube.

22 300 x g, 25°C, 00:03:00 3m

23 Resuspend the cells in 1 mL of Day 12 media with rock inhibitor and Count the cells.

24 On D12 change to D12 terminal media for final differentiation, change half media every 48h.

25 On D35 change to long-term maintaining media.

26 Protocol Media schedule
<!--td {border: 1px solid #cccccc;}br {mso-data-placement:same-cell;}-->

	A	B
	Day x--1	StemFlex
	Days 0-1	DMEM/F12+Glutamax + B27 + N2 + SB + LDN
	Days 2-3	DMEM/F12+Glutamax + B27 + N2 + SB + LDN + Purmorphamine + SAG
	Days 4-7	DMEM/F12+Glutamax + B27 + N2 + SB + LDN + Purmorphamine + SAG + CHIR
	Days 8-11	DMEM/F12+Glutamax + B27 + N2 + LDN + CHIR
	Days 12-21	DMEM/F12+Glutamax + B27 + N2 + BDNF + GDNF + DAPT+ Ascorbic Acid + dCAMP
	Days 22-35	DMEM/F12+Glutamax + B27 + N2 + 0.5(BDNF + GDNF) + DAPT + Ascorbic Acid + dCAMP
	Days 35-end	DMEM/F12+Glutamax + B27 + N2 + 0.5(BDNF + GDNF) + Ascorbic Acid