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iPSC Midbrain differentiation in 96 well plates

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Protocol status: Working

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 μ 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 μ M SB431542

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

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

D8-11 - DMEM/F-12+Glutamax + 13 B-27 minus vitamin A + 13 N-2 + 100 nM LDN193189 + 3 μ M 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 μ M DAPT + 0.1 μ M 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 μ M Ascorbic Acid + 10 μ M 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 μ M Ascorbic Acid



iPSCs plating for differentiation

8m

- 1 Passage 1×10^6 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 .
- 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
- 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.

5m

3m

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