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Midbrain organoid differentiation in spinner flasks.

In 1 collection

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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 spheres making
StemFlex medium + 10 µM Y-27632 + 1:100 Pen/Strep

Media composition for diferenciation
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 aggregation in spinner flasks

8m

1 Passage iPSC lines to four 10-cm dishes to generate 40X10^6 cells.

2 Prepare 120 mL of StemFlex per flask with rock inhibitor (refer to material section) and add 115 to the spinner flask.

3 Place the spinner flask on the stir plate and set to 65 rpm.

4 Wait for the cells to reach 80% confluence and seed iPSC 40X10^6 cells into a spinner flask.

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

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

7 300 x g, 25°C, 00:03:00 3m

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

9 Put the cells in the correct cell concentration and add to 5 mL of StemFlex with rock inhibitor.

10 From the main opening of the spinner flask add the cells and plate back on the stir plate.

11 Every other day change 60 mL of media using StemFlex until the spheres reach 300 µM.

Midbrain Differentiation

1w 5d

12 On the starting day D0, filter the spheres in a 300 µM to 500µM range.

13 Aspirate all the media in the flask and replace by D0-D1 media (refer to materials). Add the filtered spheres and place the spinner flask back on the stir plate.

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

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

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

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

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

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

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

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

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

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

24 D11 change half the media with D8-11 medium.

25 On D12 change to D12 terminal media for final differentiation. From this point on the organoids can be transfer to low attachment plates and place on shaker.

26 On D35 change to long-term maintaining media.

27 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