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Midbrain organoid differentiation in spinner flasks.

gustavo.parfitt1

¹Icahn School of Medicine



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Ahfeldt Lab

gustavo.parfitt

ABSTRACT

Midbrain differentiation protocol using spinner flasks.

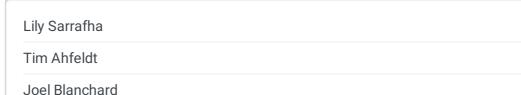
PROTOCOL CITATION

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protocols.io

https://protocols.io/view/midbrain-organoid-differentiation-in-spinner-flask-cgretv3e

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MATERIALS TEXT

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 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 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 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 + $0.2 \mu M$ Ascorbic Acid + $10 \mu M$ DAPT + $0.1 \mu M$ 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 aggregation in spinner flasks

8m

- 1 Passage iPSC lines to four 10-cm dishes to generate 40X10⁶ 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 40X10⁶ cells into a spinner flask.
- 5 For the passaging add $\square 3$ mL of Accutase for $\bigcirc 00:05:00$.

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 $\blacksquare 60 \text{ mL}$ of media using StemFlex until the spheres reach 300 μM .

Midbrain Differentiation

- 1w 5d
- 19 On the starting day D0, filter the spheres in a 300 μ M to 500 μ M range.
- 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.

- 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

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Α	В
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