Sep 14, 2022

Midbrain organoid generation from mfNPC

Rachel Bates[1]
[1]UCL

dx.doi.org/10.17504/protocols.io.6qpvr4x1pgmk/v1

rachel.bates

ABSTRACT

This protocol describes our method for the differentiation of human floor plate neural progenitor cells into human midbrain-like organoids (hMLOs). This protocol has been developed using a combination of several published protocols.

Adapted from

Jo J, Xiao Y, Sun AX, Cukuroglu E, Tran HD, Göke J, Tan ZY, Saw TY, Tan CP, Lokman H, Lee Y, Kim D, Ko HS, Kim SO, Park JH, Cho NJ, Hyde TM, Kleinman JE, Shin JH, Weinberger DR, Tan EK, Je HS, Ng HH (2016). Midbrain-like Organoids from Human Pluripotent Stem Cells Contain Functional Dopaminergic and Neuromelanin-Producing Neurons.. Cell stem cell.
https://doi.org/10.1016/j.stem.2016.07.005

Mohamed NV, Sirois J, Ramamurthy J, Mathur M, Lépine P, Deneault E, Maussion G, Nicouleau M, Chen CX, Abdian N, Soubannier V, Cai E, Nami H, Thomas RA, Wen D, Tabatabaei M, Beitel LK, Singh Dolt K, Karamchandani J, Stratton JA, Kunath T, Fon EA, Durcan TM (2021). Midbrain organoids with an SNCA gene triplication model key features of synucleinopathy.. Brain communications.
https://doi.org/10.1093/braincomms/fcab223

DOI
dx.doi.org/10.17504/protocols.io.6qpvr4x1pgmk/v1

PROTOCOL CITATION

Rachel Bates 2022. Midbrain organoid generation from mfNPC. **protocols.io**
https://protocols.io/view/midbrain-organoid-generation-from-mfnpc-cgastsee

FUNDERS ACKNOWLEDGEMENT

ASAP
Grant ID: ASAP2022

CREATED

Sep 07, 2022

LAST MODIFIED

Sep 14, 2022

PROTOCOL INTEGER ID

69682

Day 0

1 Human floor plate neuronal progenitor cells (mfNPC) were derived using Smits 2019 protocol. They were maintained for a minimum of 5 passages before being used to generate organoids in maintenance medium.

Fedele S, Collo G, Behr K, Bischofberger J, Müller S, Kunath T, Christensen K, Gündner AL, Graf M, Jagasia R, Taylor V (2017). Expansion of human midbrain floor plate progenitors from induced pluripotent stem cells increases dopaminergic neuron differentiation potential.. Scientific reports.
https://doi.org/10.1038/s41598-017-05633-1

Smits LM, Reinhardt L, Reinhardt P, Glatza M, Monzel AS, Stanslowsky N, Rosato-Siri MD, Zanon A, Antony PM, Bellmann J, Nicklas SM, Hemmer K, Qing X, Berger E, Kalmbach N, Ehrlich M, Bolognin S, Hicks AA, Wegner F, Sterneckert JL, Schwamborn JC (2019). Modeling Parkinson's disease in midbrain-like organoids.. NPJ Parkinson's disease.
https://doi.org/10.1038/s41531-019-0078-4

1.1 mfNPC maintenance medium

[M]50 % volume	☒DMEM/F-12 Thermo Fisher Catalog #11320033
[M]50 % volume	☒Neurobasal Plus Medium Gibco, ThermoFisher Catalog #A3582901
1:50	☒N21-MAX Media Supplement (50X) R&D Systems Catalog #AR008
1:100	☒N-2 max supplement R&D Systems Catalog #AR009
[M]1 % volume	☒Glutamax (100x) Gibco - Thermo Fischer Catalog #35050-061
[M]1 % volume	☒MEM, NEAA, no glutamine Thermo Fisher Catalog #10370021
[M]10 micromolar (µM)	☒SB431542 Cell Signaling Technology Catalog #14775

SAG 1 mg **Stemcell**
0.5 micromolar (µM) **Technologies Catalog #73412**
250 millimolar (mM)
Stemolecule LDN-193189 **Stemgent - Bio-connect Catalog #04-0074**
L-Ascorbic acid **Sigma**
200 micromolar (µM) **Aldrich Catalog #A4403**
CHIR99021 **R&D**
3 micromolar (µM) **Systems Catalog #4423**

2 mfNPCs were detached using accutase at **37 °C** for **00:03:00** . 3m

3 Re-suspend cells in d0 induction medium and plate 9,000 cell/well in ultra-low attachment U-bottomed 96 well plates.

BIOFLOAT plate from Facellitate work best for us compared to Corning or Nunc at producing uniform EBs.

Day 2

4 Change the medium to mfNPC medium supplemented with **0.0001 mg/mL**
FGF8 **Novus**
Biologicals Catalog #423-F8-025 **0.0001 mg/mL**
recombinant human SHH protein **Qkine** being careful not to touch the organoid.

Day 4

5 Change the medium to patterning I medium adding **300 µL** per well.

5.1 **patterning I medium**

DMEM/F-12 **Thermo**
50 % volume **Fisher Catalog #11320033**
50 % volume
Neurobasal Plus Medium **Gibco,**
ThermoFisher Catalog #A3582901
1:50
N21-MAX Media Supplement (50X) **R&D**
Systems Catalog #AR008
N-2 max supplement **R&D**
1:100 **Systems Catalog #AR009**

Citation: Rachel Bates Midbrain organoid generation from mfNPC https://dx.doi.org/10.17504/protocols.io.6qpvr4x1pgmk/v1

	☒ Glutamax (100x) **Gibco - Thermo**
[M] **1 % volume**	**Fischer Catalog #35050-061**
	☒ MEM, NEAA, no glutamine **Thermo**
[M] **1 % volume**	**Fisher Catalog #10370021**
	☒ SAG 1 mg **Stemcell**
[M] **0.5 micromolar (µM)**	**Technologies Catalog #73412**
	☒ L-Ascorbic acid **Sigma**
[M] **200 micromolar (µM)**	**Aldrich Catalog #A4403**
	☒ CHIR99021 **R&D**
[M] **3 micromolar (µM)**	**Systems Catalog #4423**
	☒ FGF8 **Novus**
[M] **0.0001 mg/mL**	**Biologicals Catalog #423-F8-025**
[M] **0.0001 mg/mL**	☒ recombinant human SHH protein **Qkine**

Day 6

6 Change medium to patterning II medium with reduced CHIRR adding **300 µL** per well.

6.1 Patterning II medium

	☒ DMEM/F-12 **Thermo**
[M] **50 % volume**	**Fisher Catalog #11320033**
[M] **50 % volume**	
☒ Neurobasal Plus Medium **Gibco, ThermoFisher Catalog #A3582901**	
1:50	
☒ N21-MAX Media Supplement (50X) **R&D Systems Catalog #AR008**	
	☒ N-2 max supplement **R&D**
1:100	**Systems Catalog #AR009**
	☒ Glutamax (100x) **Gibco - Thermo**
[M] **1 % volume**	**Fischer Catalog #35050-061**
	☒ MEM, NEAA, no glutamine **Thermo**
[M] **1 % volume**	**Fisher Catalog #10370021**
	☒ L-Ascorbic acid **Sigma**
[M] **200 micromolar (µM)**	**Aldrich Catalog #A4403**
	☒ CHIR99021 **R&D**
[M] **0.7 micromolar (µM)**	**Systems Catalog #4423**
	☒ FGF8 **Novus**
[M] **0.0001 mg/mL**	**Biologicals Catalog #423-F8-025**
[M] **0.0001 mg/mL**	☒ recombinant human SHH protein **Qkine**

7 ☒ Cultrex Reduced Growth Factor Basement Membrane Extract **R&D Systems Catalog #343301001** Thaw

Overnight at **4 °C** ready for next day.

Day 8 30m

Citation: Rachel Bates Midbrain organoid generation from mfNPC https://dx.doi.org/10.17504/protocols.io.6qpvr4x1pgmk/v1

8 Carefully remove as much medium as possible from each well being careful not to touch the organoid.

9 Add **15 µL** of
Cultrex Reduced Growth Factor Basement Membrane Extract **R&D Systems Catalog #343301001**
to each well and return to **37 °C** incubator for **00:30:00** .
30m

10 Carefully add **300 µL** of tissue induction medium to each well.

Some organoids may float others with remain attached to plate, this does not affect the organoid.

10.1 Tissue induction medium

[M] **100 % volume**
Neurobasal Plus Medium **Gibco, ThermoFisher Catalog #A3582901**

1:50
N21-MAX Media Supplement (50X) **R&D Systems Catalog #AR008**

1:100
N-2 max supplement **R&D Systems Catalog #AR009**

[M] **1 % volume**
Glutamax (100x) **Gibco - Thermo Fischer Catalog #35050-061**

[M] **1 % volume**
MEM, NEAA, no glutamine **Thermo Fisher Catalog #10370021**

[M] **200 micromolar (µM)**
L-Ascorbic acid **Sigma Aldrich Catalog #A4403**

[M] **0.0001 mg/mL**
FGF8 **Novus Biologicals Catalog #423-F8-025**

[M] **0.0001 mg/mL**
recombinant human SHH protein **Qkine**

[M] **0.00025 mg/mL**
Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane **Sigma – Aldrich Catalog #L2020**

[M] **0.025 mg/mL**
Insulin **Sigma Aldrich Catalog #I2643-25MG**

Day 9 30m

11 Using sterilised scissors or a scalpel blade cut a pasteur pipette at the widest point.

this will provide a sufficient bore to pick up the embedded organoid without damaging the cultrex.

Citation: Rachel Bates Midbrain organoid generation from mfNPC https://dx.doi.org/10.17504/protocols.io.6qpvr4x1pgmk/v1

12 Either add **2 mL** of Tissue induction medium to Ultra-low attachment 6-well plate **Corning Catalog #CLS3471** or add **500 µL** to Ultra-low attachment 48 well plate.

13 using the cut pasteur pipette carefully pick up each organoid and move to new plate. For 6 well plate add up to 6 organoids per well or 1 per well in the 48-well plate.

14 Incubate plates at **37 °C** on a orbital shaker set to **70 rpm** .

Day 10

15 Change medium to Differentiation medium.

15.1 **Differentiation medium**

[M]**100 % volume**	Neurobasal Plus Medium **Gibco, ThermoFisher Catalog #A3582901**
1:50	N21-MAX Media Supplement (50X) **R&D Systems Catalog #AR008**
1:100	N-2 max supplement **R&D Systems Catalog #AR009**
[M]**1 % volume**	Glutamax (100x) **Gibco - Thermo Fischer Catalog #35050-061**
[M]**1 % volume**	MEM, NEAA, no glutamine **Thermo Fisher Catalog #10370021**
[M]**200 micromolar (µM)**	L-Ascorbic acid **Sigma Aldrich Catalog #A4403**
[M]**125 micromolar (µM)**	Db-cAMP (dibutyryl-cyclic AMP) **Sigma Catalog #D0627**
10ng/mL	BDNF **Qkine Catalog #QK050**
10ng/mL	GDNF **Qkine Catalog #QK051**

16 After day 10 perform a 75% medium change every 2-3 days.

17

Citation: Rachel Bates Midbrain organoid generation from mfNPC https://dx.doi.org/10.17504/protocols.io.6qpvr4x1pgmk/v1