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Midbrain organoid generation from mfNPC

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Protocol status: Working

We use this protocol and it's working

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ASAP

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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

Citation

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>

LINK

Citation

Mohamed NV, Sirois J, Ramamurthy J, Mathur M, Lépine P, Deneault E, Maussion G, Nicouveau 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>

LINK



Protocol materials

- ✕ recombinant human SHH protein **Qkine**
- ✕ FGF8 **Novus Biologicals Catalog #423-F8-025**
- ✕ Cultrex Reduced Growth Factor Basement Membrane Extract **R&D Systems Catalog #343301001**
- ✕ Ultra-low attachment 6-well plate **Corning Catalog #CLS3471**
- ✕ Cultrex Reduced Growth Factor Basement Membrane Extract **R&D Systems Catalog #343301001**
- ✕ DMEM/F-12 **Thermo Fisher Catalog #11320033**
- ✕ Stemolecule LDN-193189 **Stemgent - Bio-connect Catalog #04-0074**
- ✕ L-Ascorbic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4403**
- ✕ N21-MAX Media Supplement (50X) **R&D Systems Catalog #AR008**
- ✕ SB431542 **Cell Signaling Technology Catalog #14775**
- ✕ CHIR99021 **R&D Systems Catalog #4423**
- ✕ Neurobasal Plus Medium **Gibco - Thermo Fisher Scientific Catalog #A3582901**
- ✕ MEM, NEAA, no glutamine **Thermo Fisher Catalog #10370021**
- ✕ SAG 1 mg **STEMCELL Technologies Inc. Catalog #73412**
- ✕ N-2 max supplement **R&D Systems Catalog #AR009**
- ✕ Glutamax (100x) **Gibco - Thermo Fisher Scientific Catalog #35050-061**
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☒ Insulin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I2643-25MG**

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☒ Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2020**

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

☒ FGF8 **Novus Biologicals Catalog #423-F8-025**

☒ Neurobasal Plus Medium **Gibco - Thermo Fisher Scientific Catalog #A3582901**

☒ N-2 max supplement **R&D Systems Catalog #AR009**

☒ MEM, NEAA, no glutamine **Thermo Fisher Catalog #10370021**

☒ BDNF **Qkine Catalog #QK050**

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

☒ Glutamax (100x) **Gibco - Thermo Fisher Scientific Catalog #35050-061**

☒ Db-cAMP (dibutyryl-cyclic AMP) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D0627**

☒ GDNF **Qkine Catalog #QK051**

☒ L-Ascorbic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4403**

Troubleshooting



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.

Citation

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>

LINK

Citation

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, Sternecker 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>

LINK

1.1 mfNPC maintenance medium

[M] 50 % volume

 DMEM/F-12 Thermo Fisher Catalog #11320033

[M] 50 % volume

 Neurobasal Plus Medium Gibco - Thermo Fisher Scientific 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 Fisher Scientific Catalog #35050-061**

[M] 1 % volume

 MEM, NEAA, no glutamine **Thermo Fisher Catalog #10370021**[M] 10 micromolar (μM)  SB431542 **Cell Signaling Technology Catalog #14775**[M] 0.5 micromolar (μM) SAG 1 mg **STEMCELL Technologies Inc. Catalog #73412**

[M] 250 millimolar (mM)

 Stemolecule LDN-193189 **Stemgent - Bio-connect Catalog #04-0074**[M] 200 micromolar (μM) L-Ascorbic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4403**[M] 3 micromolar (μM)  CHIR99021 **R&D 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.

Note

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 [M] 0.0001 mg/mL  FGF8 **Novus Biologicals Catalog #423-F8-025** [M] 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 μ L per well.

5.1 patterning I medium

[M] 50 % volume

 DMEM/F-12 Thermo Fisher Catalog #11320033

[M] 50 % volume

 Neurobasal Plus Medium Gibco - Thermo Fisher Scientific 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 Fisher Scientific Catalog #35050-061

[M] 1 % volume

 MEM, NEAA, no glutamine Thermo Fisher Catalog #10370021

[M] 0.5 micromolar (μ M)

 SAG 1 mg STEMCELL Technologies Inc. Catalog #73412

[M] 200 micromolar (μ M)

 L-Ascorbic acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4403

[M] 3 micromolar (μ M)  CHIR99021 R&D Systems Catalog #4423

[M] 0.0001 mg/mL  FGF8 Novus 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 CHIR99021 adding  300 μ L per well.

6.1 Patterning II medium



[M] 50 % volume

DMEM/F-12 Thermo Fisher Catalog #11320033

[M] 50 % volume

Neurobasal Plus Medium Gibco - Thermo Fisher Scientific 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 Fisher Scientific Catalog #35050-061

[M] 1 % volume

MEM, NEAA, no glutamine Thermo Fisher Catalog #10370021

[M] 200 micromolar (μM)

L-Ascorbic acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4403

[M] 0.7 micromolar (μM) CHIR99021 R&D Systems Catalog #4423

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

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

7 Thaw

Cultrex Reduced Growth Factor Basement Membrane Extract R&D Systems Catalog #343301001

Overnight at 4 °C ready for next day.

Day 8

30m

8 Carefully remove as much medium as possible from each well being careful not to touch the organoid.**9** Add 15 μL of

30m

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 .

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

**Note**

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 - Thermo Fisher Scientific 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 Fisher Scientific Catalog #35050-061**

[M] 1 % volume

⊗ MEM, NEAA, no glutamine **Thermo Fisher Catalog #10370021**

[M] 200 micromolar (μM)

⊗ L-Ascorbic acid **Merck MilliporeSigma (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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2020**

[M] 0.025 mg/mL

⊗ Insulin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I2643-25MG**

Day 9**30m**

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

**Note**

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

- 12 Either add  2 mL of Tissue induction medium to  Ultra-low attachment 6-well plate **Corning Catalog #CLS3471** or add  500 μ L 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 - Thermo Fisher Scientific 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 Fisher Scientific Catalog #35050-061**

[M] 1 % volume

 MEM, NEAA, no glutamine **Thermo Fisher Catalog #10370021**

[M] 200 micromolar (μ M)

 L-Ascorbic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4403**



[M] 125 micromolar (μM)

 Db-cAMP (dibutyryl-cyclic AMP) **Merck MilliporeSigma (Sigma-Aldrich) 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

Citations

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. Midbrain-like Organoids from Human Pluripotent Stem Cells Contain Functional Dopaminergic and Neuromelanin-Producing Neurons.

<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. Midbrain organoids with an SNCA gene triplication model key features of synucleinopathy.

<https://doi.org/10.1093/braincomms/fcab223>

Step 1

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