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dopaminergic neurons from iPSC

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Protocol status: Working

We use this protocol and it's working

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Funders Acknowledgements:

Asap



Abstract

This protocol describes our method for the differentiation of human floor plate neural progenitor cells into human midbrain-dopaminergic neurons. 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, 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>

LINK



Protocol materials

- ☒ 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**
- ☒ FGF8 **Novus Biologicals Catalog #423-F8-025**
- ☒ N21-MAX Media Supplement (50X) **R&D Systems Catalog #AR008**
- ☒ SAG 1 mg **STEMCELL Technologies Inc. Catalog #73412**
- ☒ CHIR99021 **R&D Systems Catalog #4423**
- ☒ recombinant human SHH protein **Qkine**
- ☒ MEM, NEAA, no glutamine **Thermo Fisher Catalog #10370021**
- ☒ L-Ascorbic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A4403**
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- ☒ recombinant human SHH protein **Qkine**
- ☒ DMEM/F-12 **Thermo Fisher Catalog #11320033**



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

✕ CHIR99021 **R&D Systems Catalog #4423**

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

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

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

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

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

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

✕ recombinant human SHH protein **Qkine**

✕ 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**

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

✕ recombinant human SHH protein **Qkine**

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 dopaminergic neurons.

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 0.1×10^5 cells per well 12 well plate coated with Ultimatrix for 1 hour beforehand.

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** .



Day 4

- 5 Change the medium to patterning I medium adding  1 mL 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 CHIRR adding  1 mL 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**

Day 8

30m

7 Carefully add 1 mL of tissue induction medium to each well.

7.1 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

8 Add  2 mL of Tissue induction medium to 12 well plate.

9 Incubate plates at  37 °C .

Day 10

10 Change medium to Differentiation medium.

10.1 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 (μM)

 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

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

12 Use neurons after a further 45 days differentiation.

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>

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