

Nov 14, 2025

🌐 Improved differentiation of KOLF2.1J hiPSC to midbrain dopaminergic neurons informed by multiomics

🔗 Forked from [Differentiation of KOLF2.1J hiPSC to midbrain dopaminergic neurons](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.3byl46z48go5/v1>

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SOX6 mDA differentiation



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Protocol Citation: Guochang Lyu, Ernest Arenas 2025. Improved differentiation of KOLF2.1J hiPSC to midbrain dopaminergic neurons informed by multiomics. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.3byl46z48go5/v1>

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Protocol status: Working

We use this protocol and it's working

Created: September 09, 2025

Last Modified: November 14, 2025

Protocol Integer ID: 226841

Keywords: representative markers for ventral midbrain dopaminergic neuron, ventral midbrain dopaminergic neuron, mature midbrain dopaminergic neuron, dopaminergic neuron, mature midbrain, ventral identity, neuronal activity, sox6, resultant cell, multiomic

Abstract

This protocol was used to differentiate KOLF2.1J cells into mature midbrain dopaminergic neurons with a more ventral identity. The resultant cells show representative markers for ventral midbrain dopaminergic neurons such as EN1 or SOX6, as well as neuronal activity and other canonical markers.

Protocol materials

- ☒ Geltrex[®]; hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix **Thermo Fisher Catalog #A1569601**
- ☒ Laminin-511 **Biolamina Catalog #LN511**
- ☒ Essential 8 Flex complete medium (E8) **Gibco - Thermo Fisher Scientific Catalog #A2858501**
- ☒ L-Glutamine **Gibco - Thermo Fisher Scientific Catalog #25030024**
- ☒ Neurobasal™ Medium **Gibco - Thermo Fisher Scientific Catalog #21103049**
- ☒ N2 supplement **Gibco - Thermo Fisher Scientific Catalog #17502048**
- ☒ B27 supplement minus vitamin A **Gibco - Thermo Fisher Scientific Catalog #12587010**
- ☒ Human Recombinant BDNF **STEMCELL Technologies Inc. Catalog #78005**
- ☒ Human Recombinant GDNF **STEMCELL Technologies Inc. Catalog #78058**
- ☒ Tgf beta 3 (human) Recombinant Protein **Invitrogen - Thermo Fisher Catalog #RP8600**
- ☒ Ascorbic Acid 500 mg **STEMCELL Technologies Inc. Catalog #72132**
- ☒ Dibutyryl-cAMP **STEMCELL Technologies Inc. Catalog #73884**
- ☒ Y-27632 dihydrochloride **Tocris Catalog #1254**
- ☒ Poly-L-Ornithine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P4957**
- ☒ Stemolecule LDN-193189 **Stemgent - Bio-connect Catalog #04-0074**
- ☒ Stemolecule™ SB431542 **Reprocell Catalog #04-0010-10**
- ☒ Purmorphamine **Tocris Catalog #4551**
- ☒ CHIR99021 **R&D Systems Catalog #4423**

Troubleshooting

Pre-Differentiation (iPSC Expansion & Seeding)

1 Day -2 (Two Days Before Differentiation Start):

Coat culture plates with:

- Geltrex (Life Technologies)

 Geltrex[®]; hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix **Thermo Fisher Catalog #A1569601**

- Laminin-511 (Biolamina)  Laminin-511 Biolamina Catalog #LN511

2 **Seed iPSCs:**

- Plate **adapted iPSCs at a density of 200,000 cells/cm²** onto the coated plates. Maintain cells in Essential 8 Flex (Thermo Fisher Scientific) medium

 Essential 8 Flex complete medium (E8) **Gibco - Thermo Fisher Scientific Catalog #A2858501**

. Continue until cells reach ~80% confluency.

Initiation of Differentiation

3 Day 0: Start of Differentiation:

Culture Medium: Switch to **Neurobasal/N2/B27 medium** supplemented with **2 mM L-glutamine** (Invitrogen).

 Neurobasal[™] Medium **Gibco - Thermo Fisher Scientific Catalog #21103049**

 N2 supplement **Gibco - Thermo Fisher Scientific Catalog #17502048**

 B27 supplement minus vitamin A **Gibco - Thermo Fisher Scientific Catalog #12587010**

 L-Glutamine **Gibco - Thermo Fisher Scientific Catalog #25030024**

4 **Small Molecule Additives (Day 0–11):**

- LDN193189 – 250 nM (Day 0–7)

 Stemolecule LDN-193189 **Stemgent - Bio-connect Catalog #04-0074**

- SB431542 – 10 μM (Day 0–7)

 Stemolecule[™] SB431542 **Reprocell Catalog #04-0010-10**

- Purmorphamine – 2 μM (Day 0–7)  Purmorphamine **Tocris Catalog #4551**

- CHIR99021 – 0.7 μM (Day 0–3), increase to 7.5 μM from Day 4–14

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

- Y27632 – 10 μM (Day 0–1 only)  Y-27632 dihydrochloride **Tocris Catalog #1254**

Replating Plates



- 5 Day 14: Replating. Dissociate into single cells.
- 6 Replate at **800,000 cells/cm²** on **Poly-L-ornithine+LN511-coated plates**. Add **10 μM Y27632** for **48 hours** post-replating.
- ☒ Laminin-511 Biolamina Catalog #LN511
 - ☒ Poly-L-Ornithine Merck MilliporeSigma (Sigma-Aldrich) Catalog #P4957
 - ☒ Y-27632 dihydrochloride Tocris Catalog #1254

Differentiation Media Transition

- 7 Day 10–28: Switch to Differentiation Media.
- Neurobasal / B27 / L-glutamine base, plus:
 - ☒ Neurobasal™ Medium Gibco - Thermo Fisher Scientific Catalog #21103049
 - ☒ B27 supplement minus vitamin A Gibco - Thermo Fisher Scientific Catalog #12587010
 - ☒ L-Glutamine Gibco - Thermo Fisher Scientific Catalog #25030024
 - BDNF – 20 ng/mL
 - ☒ Human Recombinant BDNF STEMCELL Technologies Inc. Catalog #78005
 - GDNF – 20 ng/mL
 - ☒ Human Recombinant GDNF STEMCELL Technologies Inc. Catalog #78058
 - TGF-β3 – 1 ng/mL
 - ☒ Tgf beta 3 (human) Recombinant Protein Invitrogen - Thermo Fisher Catalog #RP8600
 - Ascorbic acid – 0.2 mM
 - ☒ Ascorbic Acid 500 mg STEMCELL Technologies Inc. Catalog #72132
 - Dibutyryl cAMP – 0.2 mM
 - ☒ Dibutyryl-cAMP STEMCELL Technologies Inc. Catalog #73884

Note on Supplements Withdrawal:

- **LDN193189, SB431542, and Purmorphamine** are withdrawn after **Day 7**.
- **N2 supplement** is withdrawn after **Day 10**.

Additional Small Molecule Treatments

- 8 Day 12–15: Add **GW3965** at **10 μM** to promote mDA identity.
- 9 Day 26–28: **Neurogenesis Promotion**.

Add **DAPT** at **10 μ M** to promote neuronal maturation.