Striatal Neuron Medium Kit

For differentiating Human iPSC-derived NSCs to functional striatal neurons

User Guide
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Product Information

What are included in the Striatal Neuron Medium Kit (ax0333):

- 2x 250 mL Striatal Neuron Basal Medium (Store at 4 °C upon receipt)
- 2x 7.5 mL Striatal Neuron Medium Supplement (Store at or below -20 °C upon receipt)
- 1 vial of Differentiation Supplement A (Store at 4 °C upon receipt)

What else you need for the striatal neuron differentiation procedures:

Cells:
Axol Human iPSC-derived Neural Stem Cells: ax0015, ax0016, ax0018 and/or ax0211 (Huntington’s disease patient-derived)

Growth Factors & Biochemical:
Recombinant Human Brain-derived Neurotrophic Factor (BDNF): ax139800
Recombinant Human Glia-derived Neurotrophic Factor (GDNF): ax139855
Y-27632 2HCl (ROCK Inhibitor): ax68168

Coating Solutions:
ReadySet + SureBond: ax0052
Preparation of Medium and Coating Reagents

**SureBond+ReadySet Coating Substrates**

- Upon receipt store SureBond at -80°C and store ReadySet at 4°C.
- Thaw the SureBond coating solution overnight at 4°C.
- Calculate the total surface area that requires coating.
- If using glass coverslips, clean coverslips thoroughly before coating with SureBond+ReadySet.

**ReadySet**

- Pre-coat your culture vessel with ReadySet at a volume of 250 μL per cm².
- Incubate at 37°C for 1 hour.
- Rinse the plate thoroughly **four times** using an equal volume of sterile distilled H₂O (e.g. if 250 μL of ReadySet, use 250 μL sterile distilled H₂O). During each rinse rock the dish to ensure thorough washing.
- **Do not let the ReadySet to dry out following rinses; proceed straight to coating with SureBond after the rinses.**

**SureBond**

- Dilute the SureBond stock solution (50x) in D-PBS (without calcium or magnesium) e.g. 120 μL in 6 mL.
- Coat the surface of your culture vessel with the SureBond 1x working solution. We recommend coating at a volume of 100 μL per cm².
- Incubate overnight at 37°C.
- Remove the SureBond from the culture dish prior to seeding of cells. Do not wash the culture vessel after coating with SureBond.
- **Do not let the SureBond coating dry out before seeding the cells.**
**Striatal Neuron Medium**
This medium is used for thawing the neural stem cells and as the base medium for striatal neuron differentiation.

- Upon receipt store **Striatal Neuron Basal Medium** at 4°C.
- Upon receipt store **Striatal Neuron Medium Supplement** at or below -20°C.
- When ready to use, thaw **Striatal Neuron Medium Supplement** overnight at 4°C.
- To make 250 mL **Striatal Neuron Medium**, add 7.5 mL **Striatal Neuron Medium Supplement** to the **Striatal Neuron Basal Medium** and mix well.
- Once supplemented, the Striatal Neuron Medium can be stored at 4°C for 4 weeks.

This protocol requires additional supplement and growth factors at critical time points during the differentiation, use the **Striatal Neuron Medium** you prepared as the base and add in the materials as below.

**Striatal Neuron Differentiation Medium A**
This medium is used for the first phase (day 1 to 18) of differentiation to striatal neurons.

- Prepare stock solution of **Differentiation Supplement A** by resuspending the content in the vial with 200 μL D-PBS (without calcium or magnesium) supplemented with 0.1% human or bovine serum albumin (HSA or BSA).
- The Differentiation Supplement A stock solution can be aliquoted and stored at -80°C for 3 months.
- Prepare **Striatal Neuron Differentiation Medium A** by adding 50 μL **Differentiation Supplement A stock solution** into 100 mL **Striatal Neuron Medium** (1 in 2000 dilution)
**Striatal Neuron Differentiation Medium B**

This medium is used for the second phase (day 19 to 33) of differentiation to striatal neurons.

- Prepare 100 µg/mL stock solutions of BDNF and GDNF by resuspending the lyophilized powder in D-PBS (without calcium or magnesium) supplemented with 0.1% human or bovine serum albumin (HSA or BSA).
- The growth factors can be aliquoted and stored at -80°C for 3 months.
- Prepare **Striatal Neuron Differentiation Medium B** by adding the following supplement and growth factors:

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Stock Concentration</th>
<th>Final Concentration</th>
<th>100 mL Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striatal Neuron Medium</td>
<td>1X</td>
<td>1X</td>
<td>100 mL</td>
</tr>
<tr>
<td>Differentiation Supplement A</td>
<td>2000X</td>
<td>1X</td>
<td>50 µL</td>
</tr>
<tr>
<td>Recombinant Human Brain-derived Neurotrophic Factor</td>
<td>100 µg/mL</td>
<td>10 ng/mL</td>
<td>10 µL</td>
</tr>
<tr>
<td>(BDNF) (ax139800)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recombinant Human Glia-derived Neurotrophic Factor</td>
<td>100 µg/mL</td>
<td>10 ng/mL</td>
<td>10 µL</td>
</tr>
<tr>
<td>(GDNF) (ax139855)</td>
<td></td>
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</tr>
</tbody>
</table>
Thawing and Plating the iPSC-derived Neural Stem Cells

The day before thawing the iPSC-derived NSCs:

- Coat cell culture vessels with substrates as stated on page 3
- Prepare the Striatal Neuron Medium as stated on page 4
- For each vial of NSCs to be thawed, prepare 20 ml Striatal Neuron Medium supplemented with 10 µM of Y-27632 (ROCK inhibitor).

On the day of thawing Human iPSC-derived NSCs (Day 0):

- Pre-warm the Y-27632 supplemented Striatal Neuron Medium to 37°C before use.
- Remove the spent SureBond coating solution and add an appropriate volume of the medium to the culture vessel (e.g. 2 ml per well of a 6-well plate).
- Transport the NSC vial from storage to the lab on dry ice.
- Quickly thaw the vial of cells in a 37°C water bath. Do not completely submerge the vial. Remove the vial before the last bit of ice has melted.
- Take the vial of cells to a class II biosafety cabinet, spray the vial and working surface with 70% ethanol and wiping with sterile paper towel.
- Once thawed, use a P1000 pipette to transfer the cells into a 15 mL sterile conical tube and add drop-wise 10 mL of pre-warmed Striatal Neuron Medium supplemented with Y-27632. While adding the medium, gently move the conical tube back and forth to mix the iPSC-derived NSCs. This reduces osmotic shock to the cells.
- Centrifuge cells at 200 x g for 5 minutes at room temperature.
- Carefully aspirate and discard the supernatant with a pipette.
- Using a P1000 pipette, gently resuspend the cell pellet in 2 mL of Striatal Neuron Medium supplemented with Y-27632.
- Perform a cell count.
- Plate the resuspended cells drop-wise and evenly at a recommended seeding density of 35,000 cells/cm².
- Gently rock the culture vessel back and forth to ensure an even seeding density.
- Incubate the cells at 37°C, 5% CO₂.
Differentiation of the Neural Stem Cells to Striatal Neurons

Phase 1 of striatal neuron differentiation

Day 1
- Prepare **Striatal Neuron Differentiation Medium A** as stated on page 4.
- The day after plating the NSCs, gently replace the spent medium with fresh pre-warmed Striatal Neuron Differentiation Medium A.
- Incubate the cells at 37°C, 5% CO₂.

Day 4 - 16
- Replace two-thirds of the medium with fresh, pre-warmed, 37°C **Striatal Neuron Differentiation Medium A** on day 4, 7, 10, 13 and 16.

Phase 2 of striatal neuron differentiation

Day 19 - 31
- Prepare **Striatal Neuron Differentiation Medium B** as stated on page 5.
- Gently remove and replace two-thirds of the medium with fresh, pre-warmed Striatal Neuron Differentiation Medium B.
- Replace two-thirds of the medium with fresh, pre-warmed Striatal Neuron Differentiation Medium B on day 22, 25, 28 and 31.

Day 33 and beyond
- On day 33 the iPSC-derived Striatal Neurons are **ready for assay**.
- For culturing beyond day 33, **Striatal Neuron Medium** (without Differentiation Supplement A) supplemented with 20 ng/mL BDNF and 20 ng/mL GDNF is recommended. The same medium change regime (change 2/3 of medium every third day) can be applied.
Got any questions? Need help with the User Guide?
Contact Axol Technical Support at
support@axolbio.com
Or
Call +44 (0) 1223 751051 (UK)
+1-800-678-AXOL (2965) (US Toll-free)