AXOL
Discovery Stems From Here

Striatal Neuron Medium Kit

For differentiating Human iPSC-derived NSCs to functional striatal neurons

User Guide

Version 2.0

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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): Focus Biomolecules 10-2301

Coating Solutions:

Polyethylamine (PEI): Sigma-Aldrich P3143 suggested

SureBond: ax0052

Preparation of Medium and Coating Reagents

Polyethylamine and SureBond Coating Substrates

- Upon receipt store SureBond at - 80°C and store Polyethylamine (PEI) according to the suppliers instructions.
- 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 PEI and SureBond.

PEI

- Due to its potential cytotoxicity we recommend that PEI is made-up fresh on the day of use.
- It should be diluted down to a recommended coating concentration of 0.05% according to the suppliers instructions.
- Pre-coat your culture vessel with PEI at a volume of 250 µL per cm^2.
- Incubate at 37ºC for 1 hour.
- Rinse the plate thoroughly **four times** using an equal volume of sterile distilled H_2O (e.g. if 250 µL of PEI, use 250 µL sterile distilled H_2O). During each rinse rock the dish to ensure thorough washing.
- **Do not let the PEI 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 cm^2.
- Incubate overnight at 37ºC.
- 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 - 80ºC 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:

Striatal Neuron Differentiation Medium B			
Supplement	**Stock Concentration**	**Final Concentration**	**100 mL Medium**
Striatal Neuron Medium	1X	1X	100 mL
Differentiation Supplement A	2000X	1X	50 µL
Recombinant Human Brain-derived Neurotrophic Factor (BDNF) (ax139800)	100 µg/mL	10 ng/mL	10 µL
Recombinant Human Glia-derived Neurotrophic Factor (GDNF) (ax139855)	100 µg/mL	10 ng/mL	10 µL

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/cm^2.**
- Gently rock the culture vessel back and forth to ensure an even seeding density.
- Incubate the cells at 37°C, 5% CO_2.

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% CO_2.

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?
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