

AXOL

Striatal Neurons

User guide

Version 3

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Updates to protocol:

Part	Change	Rationale	Previous Version	New Version
Product Information	Review and update to available cell lines	Cell lines discontinued	2	3
Product Information	Updates to expiry dates and storage conditions	Aligning with current processes	2	3
Product Information / Coating Cell Culture Vessel	Review and update to coating and thawing procedure	Aligning with current processes	2	3
Differentiation of axoCells Neural Stem Cells	For 96 well plate format volume increased to 200µL from day 1	To align with updates to process	2	3

This protocol and its associated products are for research use only.

Products are not for diagnostic or therapeutic use and are not to be administered to humans or animals.

Product Information

This User Guide is for the following products			
Catalog No	Product Name	Format	Storage on Arrival
ax0015/ax0004/ax0219	axoCells™ Human iPSC-derived Neural Stem Cells	≥1.5 million cells/vial	Vapour phase LN ₂ storage

Media Components - Axol					
Catalog No	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions
ax0333-a	Striatal Neuron Basal Medium	2 x 250 ml	1x	Aliquot and store at 2–8°C	N/A
ax0333-b	Striatal Neuron Medium Supplement	2 x 7.5ml	1x	Aliquot and store up to 6 months at -80°C.	Overnight at 2–8°C. Keep in the dark.
ax0333-c	Differentiation Supplement A	1 vial	N/A	-20 to -80°C	Reconstituted protein should be used immediately or stored in working aliquots at -20 for up to 3 months.
ax139800 (10µg)	Recombinant Human Brain-Derived Neurotrophic Factor (BDNF)	10µg Lyophilized Powder	N/A	-20 to -80°C	Reconstituted protein should be used immediately or stored in working aliquots at -20 for up to 6 months.
ax139855 (10µg)	Recombinant Human Glial-Derived Neurotrophic Factor (GDNF)	10µg Lyophilized Powder	N/A	-20 to -80°C	Reconstituted protein should be used immediately or stored in working aliquots at -20 for up to 6 months.
ax0053	SureBond-XF	1ml	200x	Store at 4°C until date of expiry	N/A

Lot-specific information such as specifications and quality control details are stated in the Certificate of Analysis. Expiry dates for Axol-supplied components are stated on the label. Consult the manufacturer's guidelines for expiry dates of any additional reagents.

IMPORTANT!

Axol Neural Cell Culture Media DOES NOT contain antibiotics or antifungal agents. Axol does not recommend the use of antimicrobial agents such as penicillin, streptomycin and amphotericin. Antimicrobial agents should not be necessary if proper aseptic techniques are adopted.

Process Overview

Stage	Thawing & Plating	Neuronal Differentiation	Neuronal Maturation.	Long Term Culture
	D0	D1-18	D19-31	D32+
Basal Medium	Striatal Neuron Medium			
Supplement	Y-27632.2HCl, Surebond-XF	Differentiation Supplement A, Surebond-XF	Differentiation Supplement A, BDNF, GDNF, Surebond-XF	BDNF, GDNF, Surebond-XF
Final Media	Striatal Plating Medium	Striatal Neuron Differentiation Medium A	Striatal Neuron Differentiation Medium B	Long Term Culture Medium
Coating	PDL & Surebond-XF			

Preparation of Media

Prepare all chemicals and media in a class II biosafety cabinet under sterile conditions.

Preparation of Striatal Neuron Medium

This medium is used for thawing the neuronal stem cells and as the base medium for striatal neuron differentiation.

- Upon receipt, thaw Striatal Neuron Medium Supplement ax0333-b overnight at 4°C in the dark and aliquot per your requirements.
- Store aliquots at -80°C for up to 6 months (or until the earliest expiry date of a component).
- When ready to use, thaw an aliquot of Striatal Neuron Medium Supplement overnight at 4°C.
- To make 250mL Striatal Neuron Medium, add 7.5mL Striatal Neuron Medium Supplement ax0333-b to the Striatal Neuron Basal Medium ax0333-a and mix well. Adjust volumes as required.
- Store **Striatal Neuron Medium** at 4°C for up to 4 weeks, or until the earliest expiry date of a component.

Striatal Plating Medium

- Prepare **Striatal Plating Medium** as per the table below. Once made, the **Striatal Plating Medium** must be used immediately – do not store.
- Typically, 30 mL of **Striatal Plating Medium** will be required for thawing cells and plating.

Reagent	Stock Conc.	Dilution	Final Conc.
Y-27632.2HCl	2000x	1:1000	2x
Striatal Neuron Medium (ax0333-a + ax0333-b)	1x	N/A	1x
Filter sterilise using 0.22µM PES filter prior to addition of SureBond-XF			
SureBond-XF (ax0053)	200x	1:400	0.5x

Preparation of Stock Solutions

This User Guide is for the following products			
Reagent	Stock Conc.	Reconstitution	Storage after reconstitution
Y-27632.2HCl	10mM (1000x)	<i>Sterile tissue culture-grade water</i>	3 months, -20°C
Human BDNF (ax139800-10µg)	20µg/mL (2000x)	0.05% HSA in D-PBS, 0.22µM filter sterilised	6 months, -20°C
Human GDNF (ax139855-10µg)			3 months, -20°C
Differentiation Supplement A (ax0333-c)	2000x		3 months, -20°C

Prepare all chemicals and growth factors in a Class II Biosafety Cabinet under sterile conditions.

Preparation of Differentiation Supplement A

- Prepare a 0.05% solution of HSA in Dulbecco's Phosphate-Buffered Saline (D-PBS) (without calcium or magnesium). Filter sterilise the HSA prior to use (0.2µm).
- Prepare a 2000x stock solution of Differentiation Supplement A by resuspending the contents in the vial with 200µL sterile 0.05% solution of HSA.
- We do not recommend repeated freeze-thaws of reconstituted proteins. We would recommend dilution to stock concentration and aliquoting into single-use aliquots of appropriate volumes. The Differentiation Supplement A stock solution can be aliquoted and stored at -20°C for 3 months.

Preparation of BDNF Growth Factor (ax139800)

- Prepare a 0.05% solution of HSA in Dulbecco's Phosphate-Buffered Saline (D-PBS) (without calcium or magnesium). Filter sterilise the HSA prior to use (0.2µm).
- Prepare a stock solution of BDNF by resuspending the lyophilized powder in 0.05% HSA. The recommended concentrations and dilution factors are outlined in the table above.
- We do not recommend repeated freeze-thaws of reconstituted proteins. We would recommend dilution to stock concentration and aliquoting into single-use aliquots of appropriate volumes e.g. BDNF at 10µL of 20µg/mL for 20 mL media. The growth factors can be aliquoted and stored at -20°C for up to 6 months.

Preparation of GDNF Growth Factor (ax139855)

- Prepare a 0.05% solution of HSA in Dulbecco's Phosphate-Buffered Saline (D-PBS) (without calcium or magnesium). Filter sterilise the HSA prior to use (0.2µm).
- Prepare a stock solution of GDNF by resuspending the lyophilized powder in 0.05% HSA. The recommended concentrations and dilution factors are outlined in the table above.
- We do not recommend repeated freeze-thaws of reconstituted proteins. We would recommend dilution to stock concentration and aliquoting into single-use aliquots of appropriate volumes e.g. GDNF at 10µL of 20µg/mL for 20mL media. The growth factors can be aliquoted and stored at -20°C for up to 6 months.

Coating Cell Culture Vessel

A variety of extracellular matrices (ECM) can be used for the cells, such as PEI, Polyornithine, Poly-D-lysine, Laminin or Matrigel.

Recommended well volumes for each plate type: 12-well = 500µL, 48-well = 300µL, 96-well = 100µL.

We would recommend following ECM combination for culturing Neural Stem Cells to Striatal Neurons:

PDL

- Dilute the liquid poly-D-lysine 1:1 in D-PBS (-/-) to create a 0.05 mg/ml working solution (0.5x).
- Add 300 mL per cm² of 0.5x PDL working solution (0.05 mg/mL) to the culture vessel. Incubate for 60 mins at 37°C, remove the solution through aspiration and thoroughly rinse the surface twice with D-PBS (-/-).
- Remove D-PBS (-/-) and allow the surface to dry for at least two hours before proceeding onto **SureBond-XF** coating.

SureBond-XF

- Dilute the **SureBond-XF** stock solution (200x) in D-PBS (-/-) to make 1x working solution, e.g. 30µL in 6mL.
- Coat the culture surface with 300µL per cm² of the **SureBond-XF** 1x working solution.
- Incubate for at least 4 hours at 37°C, preferably overnight.
- Remove the **SureBond-XF** from the culture vessel immediately prior to plating of cells. Do not wash the culture vessel after coating with **SureBond-XF**.
- Do not let the **SureBond-XF** coating dry out before plating the cells.

Thawing and Plating axoCells Neural Stem Cells (Day 0)

1. Pre-warm the **Striatal Plating Medium** to 37°C and then add 8mL to a sterile 15mL conical tube.
2. To thaw the axoCells neural stem cells, transfer the vial of cells from nitrogen storage with the vial buried in dry ice.
3. Remove the vial from dry ice and transfer it immediately to a 37°C water bath. Do not completely submerge the vial (only up to 2/3rd of the vial). Remove the vial before the last bit of ice has melted.
4. Do not shake the vial during thawing.
5. Spray the vial with 70% ethanol before placing it in the cell culture hood.
6. Once thawed completely, use a P1000 pipette to gently transfer the cells into the 15mL tube containing the 8mL plating media from step 2.
7. Gently wash the cryovial with 1mL of the warm **Striatal Plating Medium** and transfer this to the 15 mL tube containing the cell suspension (10 mL total).

IMPORTANT!

Do not mix the cells vigorously. Avoid generating bubbles while pipetting.

8. Centrifuge cells at **200 x g** for **5** minutes at room temperature.
9. Aspirate the supernatant carefully and gently resuspend the cell pellet in **1mL** of **Striatal Plating Medium** until they become mostly a single-cell suspension.
10. Perform a cell count. We recommend seeding axoCells Striatal Neurons at a density of **100,000 cells per cm²** for monoculture.
11. Prepare cell suspension in **Striatal Plating Medium**.
12. Remove **SureBond-XF** coating solution from the culture vessel.

Timing is critical in this step. NSC attachment is compromised if the SureBond-XF is allowed to dry. Under typical conditions, the well will begin to dry within a few minutes after aspiration of the excess SureBond-XF solution.

13. Plate the resuspended cells dropwise and evenly.
14. Gently rock the culture vessel back and forth to ensure an even seeding density.
15. Incubate the cells in a humidified incubator at 37°C, 5% CO₂.

Differentiation of axoCells Neural Stem Cells (Day 1-18)

1. On day 1, prepare **Striatal Neuron Differentiation Medium A** as per the table below and use immediately.

Reagent	Stock Conc.	Dilution	Final Conc.
Differentiation Supplement A (ax0333-c)	2000x	1:2000	1x
Striatal Neuron Medium (ax0333-a + ax0333-b)	1x	N/A	1x
Filter sterilise using 0.22µM PES filter prior to addition of SureBond-XF			
SureBond-XF (ax0053)	200x	1:400	0.5x

2. Pre-warm the **Striatal Neuron Differentiation Medium A** to 37°C.
3. Remove 90% of the spent plating media and replace with warm **Striatal Neuron Differentiation Medium A**. If culturing cells in a 96-well plate, it is recommended to increase the total well volume to 200µL.
4. Perform a half media change every other day (e.g. on days 3, 5, 7...).

Maturation of young neurons (Day 19-31)

1. Prepare **Striatal Neuron Differentiation Medium A** as per the table below and use immediately.

Reagent	Stock Conc.	Dilution	Final Conc.
Differentiation Supplement A (ax0333-c)	2000x	1:2000	1x
Striatal Neuron Medium (ax0333-a + ax0333-b)	1x	N/A	1x
Human BDNF (ax139800-10µg)	20µg/mL	1:2000	10ng/mL
Human GDNF (ax139855-10µg)			
Filter sterilise using 0.22µM PES filter prior to addition of SureBond-XF			
SureBond-XF (ax0053)	200x	1:400	0.5x

2. Pre-warm the **Striatal Neuron Differentiation Medium B** to 37°C.
3. On day 19, gently remove and replace **two-thirds** of the spent **Striatal Neuron Differentiation Medium A** with warm **Striatal Neuron Differentiation Medium B**.
4. Perform half media changes with pre-warmed **Striatal Neuron Differentiation Medium B** every 2-3 days.
5. Striatal neurons are assay ready on Day 21 of maturation. Quality control is performed at this stage to show expression of key markers by immunocytochemistry. Neurons can be cultured beyond this point as described below.

Day 31 and beyond

1. For culturing beyond day 31, **Striatal Neuron Medium** supplemented with 20ng/mL BDNF and 20ng/mL GDNF is recommended as per the table below. The same medium change regime (50% medium change every third day) can be applied.

Reagent	Stock Conc.	Dilution	Final Conc.
Striatal Neuron Medium (ax0333-a + ax0333-b)	1x	N/A	1x
Human BDNF (ax139800-10µg)	20µg/mL	1:1000	20ng/mL
Human GDNF (ax139855-10µg)			
Filter sterilise using 0.22µM PES filter prior to addition of SureBond-XF			
SureBond-XF (ax0053)	200x	1:400	0.5x

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