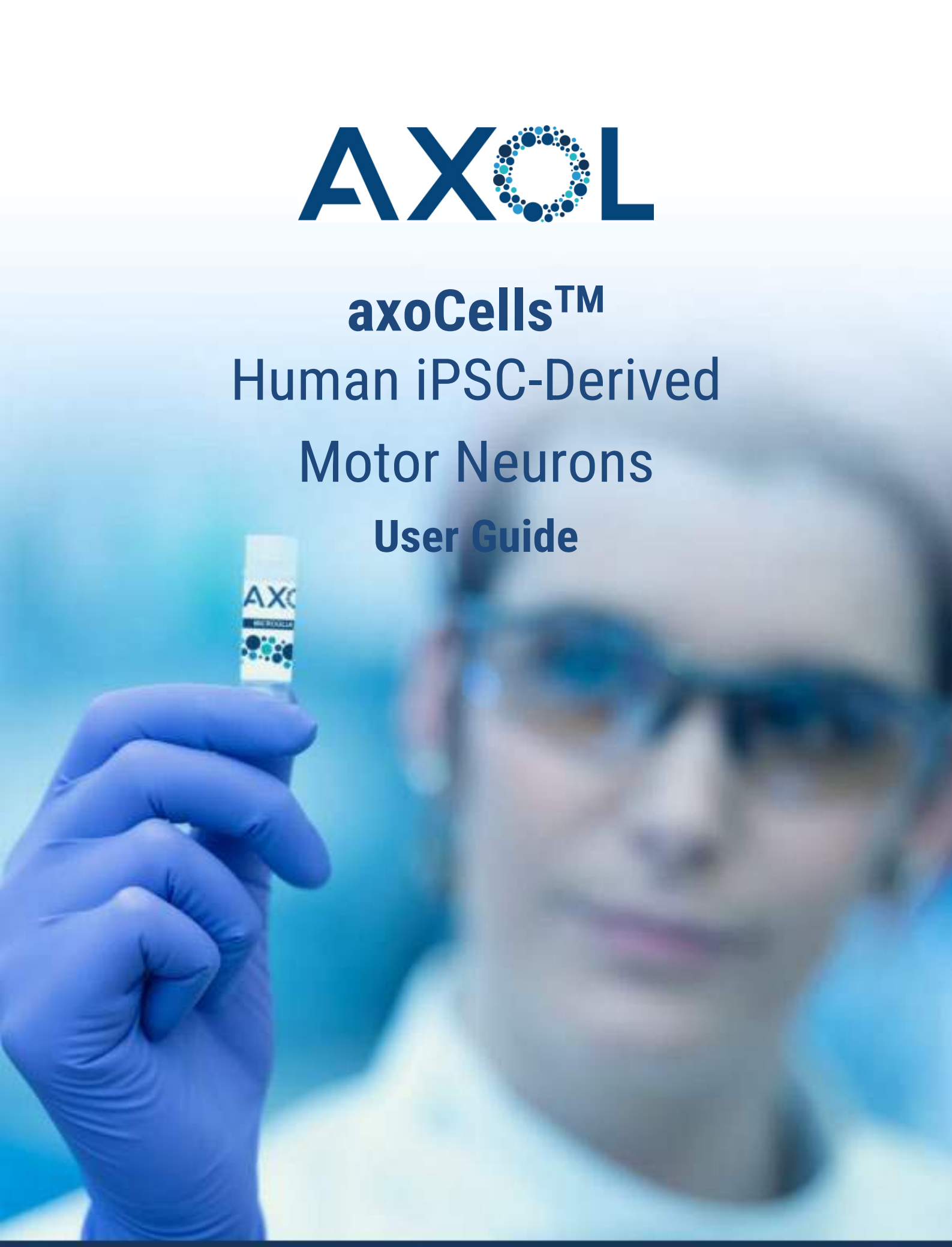
axoCells™ Human iPSC-Derived Motor Neurons

User Guide



Version 2

Table of Contents

This user guide 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.

Updates to user guide:

Part	Change	Rational	Previous version	New version
Preparation of Media and Coating Reagents	Lyophilized PDL changed to liquid PDL and change in concentration used	Enables improved cell adherence and plating consistency	Powdered PDL made up in water and used at 0.1 mg/ml	Liquid PDL diluted to 0.05mg/ml
Coating Cell Culture Ware	Coating incubation time and temperature	Enables improved cell adherence and plating consistency	Incubate plate coated with PDL for 5 mins at room temperature	Incubate plate coated with PDL for 60 mins at 37°C
Maturation of axoCells Motor Neuron Progenitors	Seeding density	Optimized cell density based on new coating reagents	150,000/cm^2	130,000 - 180,000/cm^2

Welcome

This Axol protocol is intended to be used with the full range of axoCells Human Derived Motor Neurons and the associated Axol media and reagents and the recommended and validated 3rd party reagents. Do not swap out any of the components as this will affect the success of the overall process and performance of final cell types.

For convenience and cost-effectiveness Axol have bundled the required cells, media and reagents into a range of Bundle Kits for each Motor Neuron cell-line. Additional 3rd party reagents will be required.

This protocol will allow the easy differentiation of Axol's assay-ready mature Motor Neurons in as little as 10 days.

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0076	axoCells Human iPSC-Derived Motor Neurons (unaffected male 1)	≥2 million cells/vial	N/A	Vapor Phase Nitrogen	Follow user guide	N/A
ax0186	axoCells Human iPSC-Derived Motor Neuron Bundle Kit (unaffected male 1)	Bundle Kit	N/A	Check individual components	Follow user guide	Follow user guide
ax0078	axoCells Human iPSC-Derived Motor Neurons (unaffected male 2)	≥2 million cells/vial	N/A	Vapor Phase Nitrogen	Follow user guide	N/A
ax0178	axoCells Human iPSC-Derived Motor Neuron Bundle Kit (unaffected male 2)	Bundle Kit	N/A	Check individual components	Follow user guide	Follow user guide
ax0073	axoCells Human iPSC-Derived Motor Neurons (male, C9orf72 carrying)	≥2 million cells/vial	N/A	Vapor Phase Nitrogen	Follow user guide	N/A
ax0183	axoCells Human iPSC-Derived Motor Neuron Bundle Kit (male, C9orf72 carrying)	Bundle Kit	N/A	Check individual components	Follow user guide	Follow user guide
ax0074	axoCells Human iPSC-Derived Motor Neurons (female, ALS, C9orf72)	≥2 million cells/vial	N/A	Vapor Phase Nitrogen	Follow user guide	N/A
ax0184	axoCells Human iPSC-Derived Motor Neuron Bundle Kit (female, ALS, C9orf72)	Bundle Kit	N/A	Check individual components	Follow user guide	Follow user guide
ax0735	axoCells Human iPSC-Derived Motor Neurons (female, ALS, SOD1)	≥2 million cells/vial	N/A	Vapor Phase Nitrogen	Follow user guide	N/A
ax01835	axoCells Human iPSC-Derived Motor Neuron Bundle Kit (female, ALS, SOD1)	Bundle Kit	N/A	Check individual components	Follow user guide	Follow user guide
ax0079	axoCells Human iPSC-Derived Motor Neurons (male, ALS, TDP43)	≥2 million cells/vial	N/A	Vapor Phase Nitrogen	Follow user guide	N/A
ax0189	axoCells Human iPSC-Derived Motor Neuron Bundle Kit (female, ALS, TDP43)	Bundle Kit	N/A	Check individual components	Follow user guide	Follow user guide
ax0072	Motor Neuron Maintenance Medium	200 mL	1 X	-80°C	Overnight at 4°C	Upon receipt, aliquot for long term storage at -80°C until expiration date.

ax139855 (10 µg)	Recombinant Human Glial-Derived Neurotrophic Factor (GDNF)	10 µg Lyophilized Powder	N/A	-20°C	N/A	Reconstituted protein should be used immediately or stored in working aliquots at -80°C
ax139800 (10 µg)	Recombinant Human Brain-Derived Neurotrophic Factor (BDNF)	10 µg Lyophilized Powder	N/A	-20°C	N/A	
ax139888 (5 µg)	Recombinant Human Ciliary-Neurotrophic Factor (CNTF)	20 µg Lyophilized Powder	N/A	-20°C	N/A	
ax0179	Motor Neuron Maturation Accelerator Supplement	1 mL	100x	Aliquot into required amounts and store at -80°C	Thaw at RT	Aliquots are single use

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Retinoic Acid	Sigma-Aldrich	R2625
Poly-D-Lysine	Thermo Fisher	A3890401
Y-27632 2HCl (ROCK inhibitor)	Focus Biomolecules	10-2301
Compound E	Abcam	ab142164
Vitronectin (VTN-N)	Thermo Fisher	A14700
Human Serum Albumin (HSA)	Customer's choice	Customer's choice

What's in the bundle kit?

- Frozen iPSC-derived motor neuron precursors, ≥2 million cells per vial.
- Motor Neuron Maintenance Medium
- Three Growth Factors (blue and white caps)
- Motor Neuron Maturation Accelerator (red and white cap)

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.

After extensive validation Axol has found variations in the performance of many 3rd Party reagents; both between suppliers and between different variants of the same product from the same supplier. Our internal QC validation and internal product characterization and validation experiments have all been performed using the specific recommended products outlined in this user guide. As such Axol can only guarantee the performance of Axol's products when the Axol user guide has been correctly followed, including the use of the specifically recommended Axol and 3rd Party products. Failure to correctly adhere to the Axol user guide and attempting to substitute in alternative unvalidated 3rd Party reagents may adversely affect the performance of your experiments. Therefore, any such deviations are carried out entirely at the client's own risk.

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.

Preparation of Media and Coating Reagents

Growth Factor	Stock Concentration	Final Concentration	Volume for 20mls
Recombinant Human Ciliary-Derived Neurotrophic Factor (CNTF) (ax139888)	5 µg/mL	10 ng/mL	40 µL
Recombinant Human Brain-Derived Neurotrophic Factor (BDNF) (ax139800)	10 µg/mL	5 ng/mL	10 µL
Recombinant Human Brain-Derived Neurotrophic Factor (GDNF) (ax139855)	10 µg/mL	10 ng/ml	20 µL
Motor Neuron Maturation Accelerator Supplement (ax0179)	100x	1x	200 µL
Retinoic acid	1 mM	0.5 µM	10 µL

Preparation of Growth Factors (ax139800, ax139855, ax139888)

1. Prepare a 0.05% solution of HSA in Dulbecco's Phosphate-Buffered Saline (D-PBS) (without calcium or magnesium). If necessary, filter sterilize the HSA prior to use (0.2 µm).

2. Prepare stock solutions of each growth factor by resuspending the lyophilized powder in 0.05% HSA. The recommended concentrations and dilution factors are outlined in the table above.

3. 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 50 µL of 10 µg/mL for 50 mL motor neuron media.

4. The growth factors can be aliquoted and stored at -80°C or -20°C for up to 6 months.

Motor Neuron Maturation Accelerator Supplement (ax0179)

1. Upon receipt, aliquot to your required volume and store **Motor Neuron Maturation Accelerator** supplement at -80°C protected from light. For example: for 10 mL media changes, aliquot into 100 µL aliquots.

2. When ready to use, thaw an aliquot of **Motor Neuron Maturation Accelerator** supplement overnight at 4°C in the dark. Do not refreeze after use.

Motor Neuron Maintenance Medium (ax0072)

1. Upon receipt, aliquot and store **Motor Neuron Maintenance Medium** at -80°C protected from light.

2. When ready to use, thaw an aliquot of **Motor Neuron Maintenance Medium** overnight at 4°C in the dark.

3. **Motor Neuron Maintenance Medium** requires supplementing with Compound E once thawed.

4. Prepare Compound E (ab142164) by creating a stock concentration of 1 mM in DMSO.

5. Add Compound E (ab142164) to **Motor Neuron Maintenance Medium** to create a final concentration of 0.2 µM.

6. Once made-up, **Motor Neuron Maintenance Medium** plus **Compound E** can be aliquoted and frozen for up to one month at -20°C in aliquots for future use.

Preparing "Motor Neuron Maintenance Medium – for cell plating"

Medium + Compound E

For plating out of the cells, **Motor Neuron Maintenance Medium** plus **Compound E** requires supplementing with **retinoic acid** and **Y-27632 2HCl** (Rock inhibitor) before use.

1. Prepare the **retinoic acid** by creating a stock concentration of 1 mM in DMSO.

2. Prepare the **Y-27632 2HCl** by creating a stock concentration of 10 mM in sterile water.

3. On the day of use, prepare **Motor Neuron Maintenance Medium - For Plating** by adding retinoic acid to create a final concentration of 0.1 µM (N.B this concentration is different in **Motor Neuron Maintenance Medium** (0.5 µM)) and Y-27632 2HCl to a final concentration of 10 µM.

Preparing "Motor Neuron Maintenance Medium – for maturation and maintenance"

Medium + Compound E + Retinoic acid, CNTF, GDNF, BDNF and Motor Neuron Accelerator

1. Prepare **Motor Neuron Maintenance Medium** plus **Compound E** by adding all the following factors on the day of use; **Retinoic acid, CNTF, GDNF, BDNF** and **Motor Neuron Accelerator** supplement at the concentrations stated in the table above (N.B. the concentration of retinoic acid is different (0.5 µM) from Motor Neuron Maintenance Medium - For Plating (0.1 µM).

Coating Cell Culture Ware

To enable adherence of the cells to culture ware, we recommend the following methodology. Please note the coating concentration may need to be adjusted depending on the material type of the flasks, plates or imaging slides being used.

Vitronectin + Poly-D-Lysine (PDL) Coating Solution

1. Calculate the total surface area that requires coating.
2. Dilute the liquid poly-D-lysine 1:1 in sterile water to create a 0.05 mg/ml working solution (0.5x).
3. Add 300 µL per cm^2 of 0.5x PDL 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 sterile water.
4. Remove the water and allow the surface to dry for at least two hours before proceeding onto the Vitronectin coating.
5. Dilute the Vitronectin stock solution (50x) in D-PBS (1x) (without calcium or magnesium) to make 1x working solution e.g., 10 µL in 0.5 mL.
6. Coat the surface of your culture vessel with 300 µL per cm^2 of Vitronectin 1x working solution.
7. Incubate overnight at 37°C, 5% CO_2.
8. Do not wash the culture vessel after coating with Vitronectin.
9. Do not let the Vitronectin coating dry out before plating the cells.

Thawing and Plating of axoCells Motor Neuron Progenitors

1. Prepare coating of culture vessels as described above.
2. Thaw an aliquot of **Motor Neuron Maintenance Medium** overnight at 4°C. On the day of thawing the progenitors, prepare **Motor Neuron Maintenance Medium – for plating** by adding Y-27632 2HCl to a final concentration of 10 µM and retinoic acid to a final concentration of 0.1 µM.
3. Transfer the vial of cells from storage on dry ice. Remove the vial from dry ice and transfer it to a 37°C water bath.
4. Quickly thaw the vial of cells in 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 of the ice has melted.
5. DO NOT shake the vial during thawing.
6. Take the vial of cells to a biological safety cabinet, spray the vial with 70% ethanol and wipe it down with a sterile paper towel before placing it in the cell culture hood.

7. Once thawed completely, use a P1000 pipette to transfer the cells into a 15 mL sterile conical tube containing 8 mL **Motor Neuron Maintenance Medium – For Plating**. Gently rinse the cryovial with 1 mL of fresh **Motor Neuron Maintenance Medium – For Plating** and transfer the medium with remaining cells into the 15 mL sterile conical tube.
8. **Important!** Do not mix the cells vigorously. Avoid generating bubbles while pipetting.
9. Centrifuge cells at 200 x g for 5 minutes at room temperature.
10. Carefully aspirate and discard the supernatant using a 10 mL pipette.
11. Using a P1000 pipette, gently resuspend the cell pellet in 1 mL of **Motor Neuron Maintenance Medium – For Plating** until they are in a single cell suspension.
12. Perform a cell count to ensure optimal seeding density and dilute the cell suspension as required for the chosen seeding density.
13. Recommended cell density is 100,000-180,000 cells/cm^2. Note: For high density cultures you can plate cell densities up to 240,000 cells/cm^2.
14. Remove the coating solution and add an appropriate volume of medium and cells to the culture vessel. Do not let the culture vessel dry out.
15. Incubate at 37°C, 5% CO_2 for 24 hours, then replace the cell culture medium with **Complete Motor Neuron Maintenance Medium.**

Maturation and Maintenance of axoCells Motor Neurons

1. 24h post seeding, replace 100% of the media with **Complete Motor Neuron Maintenance Medium**. Perform half media changes every Mon, Wed, Fri with fresh pre-warmed **Motor Neuron Maintenance Medium – For Maturation** and maintenance (including all the factors in the table above).

2. The motor neurons should be maintained in the above medium for a minimum of 10 days to achieve strong expression of HB9, ChAT and ISL-1. The recommended assay window for Axol's Motor Neurons is from Day 10-21 post-thaw.

iPSCs? How can we help.

Contact Axol Technical Support

at support@axolbio.com

Or

Call +44 (0) 131 651 9710