

AXOL

**axoCells™ Human iPSC-
Derived Motor Neurons**

User guide

Version 3

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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
Format of ax139888 in the Product Information table	From 20µg to 5µg	This product is supplied lyophilised at 5µg/vial.	2	3
Storage conditions once reconstituted in Product Information table	From -80°C to -20°C for BDNF, CNTF and GDNF	Growth factors are stable for 6 months at -20°C in single use aliquots.	2	3
Additional Reagents table	Update to Vitronectin used	Internal data demonstrated Vitronectin Qk120 worked effectively.	2	3
Additional Reagents table	Added DMSO, Sterile water and PBS (without Calcium or Magnesium)	Additional reagents required in the process but not listed.	2	3
Preparation of Media and Coating Reagents Section	Updated title to Preparation of Medium and Reagents and updated	More reflective of section content.	2	3

Part	Change	Rational	Previous version	New version
Preparation of Media and Coating Reagents table	Updated stock concentration of CNTF from 5µg/mL to 10µg/mL and the volume required from 40µL to 20µL	Updated to align with current process.	2	3
Page 7 Preparation of Growth factors	Amend the storage temperature once aliquoted to -20°	Updated to align with current process.	2	3
Preparation of ax0072	Changed Compound E to stock concentration of 2mM and added storage conditions of ax0072+ Comp E after thaw	Updated to align with current process and provide guidance of storage conditions for thawed medium.	2	3
Throughout document	Updated names of plating medium and maturation medium. Added table for plating and maturation medium components	Make the preparation of these components clearer.	2	3
Maturation and Maintenance of axocells Motor Neurons	24 post seeding replace 90% of the media not 100%	Updated to align with current process.	2	3

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 maturation of Axol's assay-ready mature Motor Neurons in as little as 21 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

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
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 -20°C (single use)
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)	5 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
Human Vitronectin (VTN-N)	Q-Kine	Qk120
Human Serum Albumin (HSA)	Customer's choice	Customer's choice
DMSO	Customer's choice	Customer's choice
Sterile grade water	Customer's choice	Customer's choice
PBS (without Calcium or Magnesium)	Customer's choice	Customer's choice

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 Medium and Reagents

Growth Factor	Stock Concentration	Final Concentration	Volume for 20mls
Recombinant Human Ciliary-Derived Neurotrophic Factor (CNTF) (ax139888)	10 µg/mL	10 ng/mL	20 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

Neural Plating Media Preparation of Growth Factors (ax139800, ax139855, ax139888)

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

Motor Neuron Maturation Accelerator Supplement (ax0179)

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

Motor Neuron Maintenance Medium (ax0072)

- Upon receipt store **Motor Neuron Maintenance Medium** at -80°C protected from light.
- When ready to use, thaw an aliquot of **Motor Neuron Maintenance Medium** overnight at 4°C in the dark.
- **Motor Neuron Maintenance Medium** requires supplementing with Compound E once thawed.
- Prepare Compound E (ab142164) by creating a stock concentration of 2 mM in DMSO.
- Add Compound E (ab142164) to **Motor Neuron Maintenance Medium** to create a final concentration of 0.2 µM.
- 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. Once thawed ax0072 (+ Compound E) can be stored at +4°C for up to 1 week.

Preparation of Complete Motor Neuron Plating Medium

- Prepare the **retinoic acid** by creating a stock concentration of 1 mM in DMSO.
- Prepare the **Y-27632 2HCl** by creating a stock concentration of 10 mM in sterile water.

Reagent	Stock Concentration	Final Concentration	Volume for 20mL
ax0072 + Compound E	N/A	N/A	20mL
ROCKi (Y-27632-HCl)	10mM	10µM	10µL
Retinoic Acid	1mM	0.1µM	1µL
Filter sterilize 0.2µM prior to use			

Preparation of Complete Motor Neuron Maintenance Medium

Reagent	Stock Concentration	Final Concentration	Volume for 20mL
ax0072 + Compound E	N/A	N/A	20mL
Recombinant Human Ciliary-Derived Neurotrophic Factor (CNTF) (ax139888)	10µg/mL	10ng/mL	20µL
Recombinant Human Brain-Derived Neurotrophic Factor (BDNF) (ax139800)	10µg/mL	5ng/mL	10µL
Recombinant Human Brain-Derived Neurotrophic Factor (GDNF) (ax139855)	10µg/mL	10ng/mL	20µL
Motor Neuron Maturation Accelerator Supplement (ax0179)	100x	1x	200µL
Retinoic acid	1mM	0.5µM*	10µL
Filter sterilize 0.2µM prior to use			

*The final concentration of Retinoic acid differs from that used in the Plating medium.

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 D-PBS (without calcium or magnesium) to create a 0.05 mg/ml working solution (0.5x).
3. Add 300 µL per cm² 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 D-PBS.
4. Remove the D-PBS (without calcium or magnesium) and allow the surface to dry for at least two hours before proceeding onto the Vitronectin coating.
5. Prepare the Vitronectin in Sterile water to a stock concentration of 1mg/mL.
6. Dilute the Vitronectin stock solution (1mg/mL) in D-PBS (1x) (without calcium or magnesium) to make a 10µg/mL working solution
7. Coat the surface of your culture vessel with 300 µL per cm² of Vitronectin 1x working solution.

8. Incubate overnight at 37°C, 5% CO₂.
9. Do not wash the culture vessel after coating with Vitronectin.
10. Do not let the Vitronectin coating dry out before plating the cells.

Thawing and Plating of axoCells Motor Neuron

1. Prepare coating of culture vessels as described above.
2. Thaw an aliquot of ax0072 + Compound E overnight at 4°C. On the day of thawing prepare Complete Motor Neuron Plating Medium as described in the table above.
3. Aliquot 8mL of prewarmed Complete Motor Neuron Plating Medium into a sterile 15mL conical tube.
4. 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.
5. 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.
6. DO NOT shake the vial during thawing.
7. 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.
8. Once thawed completely, use a P1000 pipette to transfer the cells into the 15 mL sterile conical tube containing 8 mL **Motor Neuron Plating Medium**. Gently rinse the cryovial with 1 mL of fresh **Motor Neuron Plating Medium** and transfer the medium with remaining cells into the 15 mL sterile conical tube.
9. **Important!** Do not mix the cells vigorously. Avoid generating bubbles while pipetting. Once cells are thawed they should be transferred to the medium quickly to avoid any effect on cell viability.
10. Centrifuge cells at 200 x g for 5 minutes at room temperature.
11. Carefully aspirate and discard the supernatant using a 10 mL pipette.
12. Using a P1000 pipette, gently resuspend the cell pellet in 1 mL of **Motor Neuron Plating Medium** until they are in a single cell suspension.
13. Perform a cell count to ensure optimal seeding density and dilute the cell suspension as required for the chosen seeding density.
14. Recommended seeding density is 100,000-180,000 cells/cm². Note: For high density cultures (e.g. MEA assays) you can plate cell densities up to 240,000 cells/cm². For neurite outgrowth and imaging-based assays 30,00-50,000 cells/cm² can be used.
15. 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.

16. Incubate at 37°C, 5% CO₂ 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, remove 90% of the media and top up to 200μL of **Complete Motor Neuron Maintenance Medium** (i.e. remove 90μL and then add 190μL fresh 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 15 onwards, although all QC and functional readouts are performed on 21 post-thaw.

Axol Bioscience

www.axolbio.com

Contact our technical support team at support@axolbio.com