axol

Human iPSC-Derived Motor Neuron Progenitors

Table of Contents

Product Information 2

Preparation of Reagents 3

Culture of Human iPSC-Derived Motor Neuron Progenitors 5

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0078	Human iPSC-Derived Motor Neuron Progenitors	>1.5 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax0071	Motor Neuron Recovery Medium	50 mL	1x	-80ºC	Overnight at 4ºC	Once thawed store aliquot at 4ºC for up to 1 week
ax0072	Motor Neuron Maintenance Medium	200 mL	1x	-80ºC	Overnight at 4ºC	Once thawed store aliquot at 4ºC for up to 1 week
ax0052	SureBond + ReadySet	SureBond 3 x 120 µL ReadySet 2 x 10 mL	SureBond 50x ReadySet 1x	SureBond -80ºC ReadySet 4ºC	SureBond Overnight at 4ºC ReadySet N/A	SureBond Store at 4ºC for up to 2 weeks ReadySet Store at 4ºC for up to 1 month
ax0041	SureBond	3 x 120 µL	50x	-80ºC	Overnight at 4ºC	Store at 4ºC for up to 2 weeks
ax0044	Unlock	25 mL	1x	Aliquot & store at -80ºC for 5 months	Overnight at 4ºC	Once thawed store aliquot at 4ºC for up to 1 week

Additional Reagents		
Product Name	**Provider**	**Catalog. No.**
Retinoic acid	Sigma-Aldrich	R2625
Brain-Derived Neurotrophic Factor (BDNF)	Peprotech	450-02
Ciliary Neurotrophic Factor (CNTF)	Peprotech	450-13
Y-27632 2HCl (ROCK inhibitor)	Selleck Chemicals	S1049

These reagents must be added fresh for each use to each aliquot of medium.

Important!
Axol Neural Cell Culture Media

DOES NOT contain antibiotics or antifungal agents. Axol Bioscience does not recommend the use of antimicrobial agents such as penicillin, streptomycin and amphotericin. Antimicrobial agents should not be necessary if proper aseptic technique is adopted.

Preparation of Reagents

Motor Neuron Recovery Medium

- Upon receipt, aliquot and store **Motor Neuron Recovery Medium** at or below **-80°C** protected from light.
- When ready to use, thaw an aliquot of **Motor Neuron Recovery Medium** overnight at **4°C** in the dark.
- **Motor Neuron Recovery Medium** requires supplementing with **retinoic acid** before use.
- Prepare the **retinoic acid** by creating a stock concentration of **1 mM** in DMSO.
- Prepare **Motor Neuron Recovery Medium** by adding **retinoic acid** to a final concentration of **0.1 µM** e.g. **5 µL** in **50 mL**.
- During thawing and passaging **Motor Neuron Recovery Medium** should be supplemented with **Y-27632 2HCl** to a final concentration of **10 µM**.

Motor Neuron Maintenance Medium

- Upon receipt, aliquot and store **Motor Neuron Maintenance Medium** at or below **-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 three compounds before use.
- Prepare **Motor Neuron Maintenance Medium** by adding the following factors fresh each time:

Growth Factor	Stock Concentration	Final Concentration	50 mL Medium
Retinoic acid	1 mM	0.5 µM	25 µL
Brain-Derived Neurotrophic Factor (BDNF)	10 µg/mL	5 ng/mL	25 µL
Ciliary Neurotrophic Factor (CNTF)	10 µg/mL	10 ng/mL	50 µL

SureBond Coating Solution (required for plating during recovery)

- Upon receipt, store **SureBond** at **-80°C**.
- Thaw the **SureBond** coating solution overnight at **4°C**.
- Calculate the total surface area that requires coating.
- Dilute the **SureBond** stock solution (50x) in Dulbecco's-PBS (1x) (D-PBS, without calcium or magnesium) to make 1x working solution 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 **200 µL per cm²**.
- Incubate your culture vessel **overnight** at **37°C**.

SureBond+ReadySet Coating Solution (required for final plating)

- Upon receipt, store **SureBond** at or below **-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.
- Pre-coat your culture vessel with **ReadySet** at a volume of **250 µL per cm²**.
- Incubate at **37ºC** for **45 minutes**.
- Wash the plate thoroughly **four times** using an equal volume of sterile ddH_2O (e.g. if 250 µL of **ReadySet**, use 250 µL sterile ddH_2O). During each wash, rock the dish to ensure thorough washing.
- Do not let the **ReadySet** dry out following washing, proceed straight to coating with **SureBond**.
- Dilute the **SureBond** stock solution (50x) in D-PBS (1x) (without calcium or magnesium) to make 1x working solution 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 **200 µL per cm²**.
- Incubate for **1 hour** at **37°C**.

Important!

Make sure that the coating does not evaporate.
Do not let the **SureBond** coating dry out before seeding the cells.
DO NOT wash the vessel after coating with **SureBond**.

Unlock (required for passaging)

- Upon receipt, aliquot and store **Unlock** at or below **-80°C** protected from light. Stored at **-80°C**, the reagent is stable for 6 months from date of manufacture.

Culture of Human iPSC-Derived Motor Neuron Progenitors

Thawing and Plating

The day before thawing **Human iPSC-Derived Motor Neuron Progenitors**

- Thaw an aliquot of **Motor Neuron Recovery Medium** overnight at **4°C**.
- Prepare culture vessels by coating with **SureBond** **overnight** prior to thawing cells.
  - **Matrigel™** can be used to coat the culture vessels, carry this out on the day of thawing at least **1 hour** before seeding the cells (prepared before seeding in accordance with manufacturer's instructions).
- T-25 flasks or 60 mm dishes are recommended for initial plating of **Human iPSC-Derived Motor Neuron Progenitors** after thawing.

On the day of thawing **Human iPSC-Derived Motor Neuron Progenitors**

- Prepare **Motor Neuron Recovery Medium** by adding **Y-27632 2HCl** to a final concentration of **10 µM** and **retinoic acid** to a final concentration of **0.1 µM**.
- Pre-warm all media and culture vessels to **37°C** before use.
- Add **4 mL** of **Motor Neuron Recovery Medium** into a 15 mL sterile conical tube.
- To thaw the cells – transfer the vial of cells from storage by transporting the vial buried in dry ice. Remove the vial from dry ice and transfer it to a **37°C** water bath.
- 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 bit of ice has melted, after 1-2 minutes.
- **Do not shake the vial during thawing.**
- Take the vial of cells to a biological safety cabinet, spraying the vial and hood thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing the vial in the hood.
- Using a P1000 pipette, gently add the cell suspension drop-wise into the 15 mL sterile conical tube containing **Motor Neuron Recovery Medium**. Gently wash the cryogenic vial with **1 mL** of warm **Motor Neuron Recovery Medium** and transfer this to the 15 mL sterile conical tube.
- Centrifuge cells at **200 x *g*** for **5 minutes** at room temperature.
- Carefully aspirate and discard the supernatant using a pipette.
- Using a P1000 pipette, gently resuspend the cell pellet in **1 mL** of **Motor Neuron Recovery Medium** until they are in a single cell suspension.
- Perform a cell count to ensure optimal seeding density.
- Remove the coating solution and add an appropriate volume of medium to the culture vessel. Do not let the culture vessel dry out.
- Plate the resuspended cells drop-wise and evenly at a density ranging from **100,000-150,000 cells/cm²**.
- Gently rock the culture vessel back and forth to ensure an even seeding density.
- Incubate the cells at **37°C**, **5% CO_2**.
- The day after plating, replace all of the medium with fresh pre-warmed, **37°C**, **Motor Neuron Recovery Medium** (without **Y-27632 2HCl**).
- **Every 2 days** replace all of the medium with fresh pre-warmed, **37°C**, **Motor Neuron Recovery Medium** (without **Y-27632 2HCl**). Passage the **Human iPSC-Derived Motor Neuron Progenitors** at **day 5-7**, depending on confluency.

Passaging and Maintenance of Human iPSC-Derived Motor Neuron Progenitors

- When the culture is 70 % confluent, it is ready to undergo passaging.

The day before passaging **Human iPSC-Derived Motor Neuron Progenitors**

- Thaw an aliquot of **Unlock** and **Motor Neuron Maintenance Medium** overnight at **4ºC** before use and store at **4ºC**.

On the day of passaging **Human iPSC-Derived Motor Neuron Progenitors**

- **Pre-Coat Culture Vessels**: Make sure to pre-coat the surface of your culture vessel that the cells will be passaged into. For final seeding, pre-coat culture vessels with **SureBond+ReadySet**.
- Prepare **Motor Neuron Maintenance Medium** by adding **Y-27632 2HCl** to a final concentration of **10 µM** and **retinoic acid** to a final concentration of **0.5 µM**, **BDNF** to a final concentration of **5 ng/mL** and **CNTF** to a final concentration of **10 ng/mL**.
- Pre-warm all media and culture vessels to **37ºC** before use.
- Remove all spent medium from cell culture vessels.
- Gently rinse the surface of the cell layer once with the D-PBS (1x) (without calcium or magnesium). We recommend using **2 mL per 10 cm²** of culture surface area.
- Discard the D-PBS.
- To detach the cells from a coating of **SureBond** use **Unlock**.
- Add **1 mL per 10cm²** of culture surface area of cold/room temperature **Unlock**. Evenly distribute it over the entire cell layer. Incubate the cells for **5 minutes** at **37°C**.
- Use a P1000 pipette to transfer the cells drop-wise into a 15 mL sterile conical tube and then gently add **four volumes** of pre-warmed, **37°C**, **Motor Neuron Maintenance Medium**. (For example if 1 mL of **Unlock** is used, then add 4 mL of the medium to stop the reaction). Gently pipette up and down a few times to disperse the medium.
- Centrifuge cells at **200 x *g*** for **5 minutes** at room temperature.
- Carefully aspirate and discard the supernatant using a pipette.
- Using a P1000 pipette, gently resuspend the cell pellet in **1 mL** of **Motor Neuron Maintenance Medium** until they are in a single cell suspension.
- Perform a cell count to ensure optimal seeding density.
- Remove the coating solution and add an appropriate volume of medium to the culture vessel. Do not let the culture vessel dry out.
- Plate the resuspended cells drop-wise and evenly at a density of **150,000-200,000 cells/cm²**.
- The day after plating, replace all of the medium with fresh pre-warmed, **37°C**, **Motor Neuron Maintenance Medium** (without **Y-27632 2HCl**).
- **Every 2 days** remove half the medium and replace with fresh pre-warmed, **37°C**, **Motor Neuron Maintenance Medium** (without **Y-27632 2HCl**).
- **Human iPSC-Derived Motor Neuron Progenitors** should be cultured for a minimum of **19-35 days**.

Note

Terminally differentiated iPSC-derived motor neurons can be cultured for up to 35 days.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
International phone **+44-1223-751-051**
US phone **+1-800-678-AXOL (2965)**

Notes

Notes

Notes

Address

Axol Bioscience Limited | Suite 3 | The Science Village |
Chesterford Research Park | Little Chesterford | Cambridgeshire | CB10 1XL

International phone

+44-1223-751-051

US phone

+1-800-678-AXOL (2965)

Email

support@axolbio.com

Web

www.axolbio.com