Human iPSC-Derived Motor Neuron Progenitors
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# Human iPSC-Derived Motor Neuron Progenitors

## Product Information

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

### Additional Reagents

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Provider</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoic acid</td>
<td>Sigma-Aldrich</td>
<td>R2625</td>
</tr>
<tr>
<td>Brain-Derived Neurotrophic Factor (BDNF)</td>
<td>Peprotech</td>
<td>450-02</td>
</tr>
<tr>
<td>Ciliary Neurotrophic Factor (CNTF)</td>
<td>Peprotech</td>
<td>450-13</td>
</tr>
<tr>
<td>Y-27632 2HCl (ROCK inhibitor)</td>
<td>Selleck Chemicals</td>
<td>S1049</td>
</tr>
</tbody>
</table>

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

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**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:

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Stock Concentration</th>
<th>Final Concentration</th>
<th>50 mL Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoic acid</td>
<td>1 mM</td>
<td>0.5 μM</td>
<td>25 μL</td>
</tr>
<tr>
<td>Brain-Derived Neurotrophic Factor (BDNF)</td>
<td>10 μg/mL</td>
<td>5 ng/mL</td>
<td>25 μL</td>
</tr>
<tr>
<td>Ciliary Neurotrophic Factor (CNTF)</td>
<td>10 μg/mL</td>
<td>10 ng/mL</td>
<td>50 μL</td>
</tr>
</tbody>
</table>

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.
Human iPSC-Derived Motor Neuron Progenitors

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 37°C for 45 minutes.
- Wash the plate thoroughly four times using an equal volume of sterile ddH₂O (e.g. if 250 μL of **ReadySet**, use 250 μL sterile ddH₂O). 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% CO₂.
- 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 4°C before use and store at 4°C.

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 37°C 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 10 cm² 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
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Notes
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