Human iPSC-Derived Mature Astrocytes
# Table of Contents

- Culture of Human iPSC-Derived Mature Astrocytes 2
- Recommendations 2
- Preparing Medium for Human iPSC-Derived Mature Astrocytes 3
- Astrocyte Maintenance Medium 3
- Coating the Culture Vessel with Matrigel™ 3
- Thawing and Plating Human iPSC-Derived Mature Astrocytes 4
Culture of Human iPSC-Derived Mature Astrocytes

<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>ax0084</td>
<td>Human iPSC-Derived Mature Astrocytes</td>
<td>1 million cells/vial</td>
<td>N/A</td>
<td>Liquid Nitrogen</td>
<td>Follow protocol</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Astrocyte Basal Medium</td>
<td>1 x 100 mL</td>
<td>1x</td>
<td>4ºC</td>
<td>N/A</td>
<td>Store at 4ºC for up to 6 months</td>
</tr>
<tr>
<td></td>
<td>Supplement A</td>
<td>1 x 9 mL</td>
<td>1x</td>
<td>4ºC</td>
<td>N/A</td>
<td>Store at 4ºC for up to 6 months</td>
</tr>
<tr>
<td></td>
<td>Supplement B</td>
<td>1 x 2 mL</td>
<td>1x</td>
<td>-20ºC</td>
<td>Overnight at 4ºC</td>
<td>Store at -20ºC for up to 6 months</td>
</tr>
<tr>
<td></td>
<td>Supplement C</td>
<td>1 x 100 mL</td>
<td>1x</td>
<td>-20ºC</td>
<td>Overnight at 4ºC</td>
<td>Store at -20ºC for up to 6 months</td>
</tr>
</tbody>
</table>

Lot-specific information such as specifications and quality control details are stated in the Certificate of Analysis.

**Recommendations**
- Recommended culture vessel coating: Matrigel™ hESC-Qualified Matrix
- Recommended cell culture medium: Astrocyte Maintenance Medium
- Recommended seeding density: 100,000-130,000 viable cells/cm²
- Recommended centrifugation speed: 400 x g for 5 minutes
- Recommended days in culture before assay: 3 days (minimum for GFAP expression in Astrocyte Maintenance Medium)

**Additional Reagents**

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Provider</th>
<th>Catalog. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrigel™ hESC-Qualified Matrix</td>
<td>BD Biosciences</td>
<td>354277</td>
</tr>
</tbody>
</table>

Matrigel™ hESC-Qualified Matrix

**Lot-specific information such as specifications and quality control details are stated in the Certificate of Analysis.**
Preparing Medium for Human iPSC-Derived Mature Astrocytes

Astrocyte Maintenance Medium
- Upon receipt aliquot the Astrocyte Basal Medium (4 x 25 mL) and store at or below 4°C protected from light.
- One day before thawing the Human iPSC-Derived Mature Astrocytes (Mature Astrocytes) thaw Supplement B and Supplement C overnight at 4°C. Aliquot Supplement B and Supplement C accordingly after preparing Astrocyte Maintenance Medium store remaining aliquots of Supplement B and Supplement C at -20°C.
- On the day of thawing the Mature Astrocytes (Day 0), transfer 25 mL of Basal Medium to a fresh 50 mL tube and add Supplements A, B and C according to the volumes outlined in Table 1. This will make Astrocyte Maintenance Medium.

Table 1: Preparation of an aliquot of Astrocyte Maintenance Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal Medium</td>
<td>25 mL</td>
</tr>
<tr>
<td>Supplement A</td>
<td>2.25 mL</td>
</tr>
<tr>
<td>Supplement B</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Supplement C</td>
<td>25 µL</td>
</tr>
</tbody>
</table>

Important!
Axol recommends: Matrigel™ hESC Qualified Matrix coating reagent for culturing the Mature Astrocytes.

Coating the Culture Vessel with Matrigel™

Cell culture vessels should be coated one day before or on the day of plating the cells. Please read the manufacturer’s manual for handling of Matrigel™ hESC-Qualified Matrix. For final seeding in the final assay format pre-coat desired vessel.
Thawing and Plating Human iPSC-Derived Mature Astrocytes

- On the day before thawing the Mature Astrocytes, prepare the Astrocyte Maintenance Medium.
- Pre-warm the culture medium and vessels to 37°C before use.
- Aliquot 5 mL of Astrocyte Maintenance Medium into a 15 mL sterile tube and pre-warm to 37°C.
- Aliquot 3 mL of Astrocyte Maintenance Medium into a 15 mL sterile tube and pre-warm to 37°C. Store the remaining media at 4°C.
- To thaw the Mature Astrocytes – transfer the vial of cells from liquid nitrogen storage with the vial buried in dry ice. Remove the vial from dry ice and transfer it immediately 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.**
- Spray the vial with 70% ethanol and wipe it with a sterile paper towel before placing it in the hood.
- Once thawed, use a P1000 pipette to immediately transfer the cells drop-wise into a 15 mL sterile conical tube containing 5 mL of pre-warmed Astrocyte Maintenance Medium. Gently wash the vial with 1 mL of Astrocyte Maintenance Medium to ensure all of the cells are transferred to the 15 mL sterile conical tube.

**Important!**

Do not mix the cells vigorously. Avoid generating bubbles.

- Centrifuge cells at 400 x g for 5 minutes at room temperature.
- Aspirate the medium carefully and resuspend the cell pellet with 1 mL of Astrocyte Maintenance Medium.
- Gently resuspend the cells until they are a single cell suspension.
- Remove 10 μL of cell suspension and mix it with 10 μL of trypan blue solution. Count the cells.
- Aspirate Matrigel™ solution from the pre-warmed 35 mm cell culture dish and immediately transfer the resuspended Mature Astrocytes into the dish. Wash the conical tube with an additional 1 mL of Astrocyte Maintenance Medium and transfer to the same culture dish. Cell density should be in range of 100,000-130,000 cells/cm².
- Plate the cells drop-wise and evenly on the culture vessels.
- Maintain the cells at 37°C, 5% CO₂ in a humidified incubator. The day of seeding the cells is Day 0.
- Monitor the cell survival and attachment the following day (Day 1).
- Replace the culture medium on Day 2 with fresh, pre-warmed Astrocyte Maintenance Medium. The medium change should be done slowly (drop-wise) pointing the pipette tip toward the wall of the cell culture vessel.
- Conduct a full medium change every 2 days with fresh, pre-warmed, 37°C, Astrocyte Maintenance Medium.
- Culture the cells for an additional 2-3 days and use for experimental assays.

**Important!**

Only pre-warm as much medium as is required.

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)