

Human iPSC-Derived Mature Astrocytes

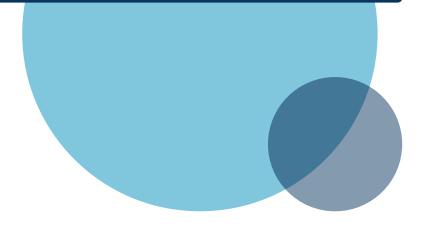






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Culture of Human iPSC-Derived Mature Astrocytes

| Catalog. No. | Product Name | Format | Stock Conc. | Storage on Arrival | Thawing Instructions | Storage Once Thawed | |
|-----------------|---|--------------------------|----------------|-----------------------|-------------------------|--------------------------------------|--|
| ax0084 | Human iPSC- Derived Mature Astrocytes | 1 million cells/ vial | N/A | Liquid Nitrogen | Follow protocol | N/A | |
| | Astrocyte Basal Medium | 1 x 100 mL | 1x | 4°C | N/A | Store at 4°C for up to 6 months | |
| | Supplement A | 1 x 9 mL | 1x | 4°C | N/A | Store at 4°C for up to 6 months | |
| | Supplement B | 1 x 2 mL | 1x | -20°C | Overnight at 4°C | Store at -20°C for up to 6 months | |
| | Supplement C | 1 x 100 mL | 1x | -20°C | Overnight at 4°C | Store at -20°C for up to 6 months | |

| Additional Reagents | | | | |
|---------------------------------|----------------|--------------|--|--|
| Product Name | Provider | Catalog. No. | | |
| Matrigel™ hESC-Qualified Matrix | BD Biosciences | 354277 | | |

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

Recommendations

Recommended culture vessel coating: Recommended cell culture medium:

Recommended seeding density:

Recommended centrifugation speed:

- Matrigel[™] hESC-Qualified Matrix Astrocyte Maintenance Medium
 - 100,000-130,000 viable cells/cm²
- Recommended days in culture before assay:
- 400 x g for 5 minutes 3 days (minimum for GFAP expression in Astrocyte Maintenance Medium)

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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

| Component | Volume |
|--------------|---------|
| Basal Medium | 25 mL |
| Supplement A | 2.25 mL |
| Supplement B | 0.5 mL |
| Supplement C | 25 µL |

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 **MatrigeI[™] 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)

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