axol

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	4ºC	N/A	Store at 4ºC for up to 6 months
	Supplement A	1 x 9 mL	1x	4ºC	N/A	Store at 4ºC for up to 6 months
	Supplement B	1 x 2 mL	1x	-20ºC	Overnight at 4ºC	Store at -20ºC for up to 6 months
	Supplement C	1 x 100 mL	1x	-20ºC	Overnight at 4ºC	Store at -20ºC 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: **Matrigel™ hESC-Qualified Matrix**
- Recommended cell culture medium: **Astrocyte Maintenance Medium**
- Recommended seeding density: **100,000-130,000 viable cells/cm^2**
- 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**)

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 **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 **37ºC** before use.
- Aliquot **5 mL** of **Astrocyte Maintenance Medium** into a 15 mL sterile tube and pre-warm to **37ºC**.
- Aliquot **3 mL** of **Astrocyte Maintenance Medium** into a 15 mL sterile tube and pre-warm to **37ºC**. Store the remaining media at 4ºC.
- 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% CO_2** 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**
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Notes

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