axol

Human iPSC-Derived Astrocyte Progenitors

Table of Contents

Culture of Human iPSC-Derived Astrocyte Progenitors

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0083	Human iPSC-Derived Astrocyte Progenitors	1 million cells/ vial	NA	Liquid Nitrogen	Follow protocol	N/A
	Astrocyte Basal Medium	2 x 50 mL	1x	4ºC	N/A	Store at 4ºC for up to 6 months
	Supplement A	2 x 4.5 mL	1 x	4ºC	N/A	Store at 4ºC for up to 6 months
	Supplement B	2 x 1 mL	1 x	-20ºC	Overnight at 4ºC	Store at -20ºC for up to 6 months
	Supplement C	2 x 50 µL	1 x	-20ºC	Overnight at 4ºC	Store at -20ºC for up to 6 months
ax0044	Unlock	1 x 25 mL	1 x	-80oC	Overnight at 4oC	Store at 4ºC for up to 1 week

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 after thawing: **100,000-130,000 viable cells/cm^2**
- Recommended seeding density after passaging: **80,000-100,000 viable cells/cm^2**
- Recommended centrifugation speed: **400 x *g*** for **5 minutes**
- Recommended days in culture before assay: **17 days** (minimum for GFAP expression in **Astrocyte Maintenance Medium**)

Preparing Medium for Human iPSC-Derived Astrocyte Progenitors

One complete medium (**Astrocyte Maintenance Medium**) is required for the maturation of **Human iPSC-Derived Astrocyte Progenitors** into mature astrocytes.

Astrocyte Maintenance Medium

- Upon receipt, aliquot and store the **Astrocyte Basal Medium** at **4°C** protected from light.
- One day before thawing the **Human iPSC-Derived Astrocyte Progenitors (Astrocyte Progenitors)**, thaw an aliquot of **Supplement B** and **Supplement C** at **4°C** overnight.
- On the day of thawing the **Astrocyte Progenitors** (**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 **Astrocyte Progenitors**.

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 thawing **Astrocyte Progenitors**, pre-coat a 35 mm dish and for passaging, pre-coat a 60 mm dish. When seeding astrocytes for the final endpoint assay, pre-coat a culture vessel appropriate to the endpoint experiment.

Thawing and Plating Human iPSC-Derived Astrocyte Progenitors

- On the day before thawing the **Astrocyte Progenitors**, 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 **2 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 **Astrocyte Progenitors** – 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 by swirling it 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 min.
- **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 **Astrocyte Progenitors** 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 the 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.
- The culture medium should be replaced **every 2 days** with fresh, pre-warmed **Astrocyte Maintenance Medium**.

Important!

Only pre-warm as much medium as is required.

Passaging and Maturation of Human iPSC-Derived Astrocyte Progenitors

Before passaging **Astrocyte Progenitors**, the cell culture vessels should be coated one day before or on the day of passaging the cells. Prepare **Matrigel™**-coated 60 mm dishes for the first passage.

On **Day 7**, the astrocytes in the 35 mm dish will be fully confluent. Passage the cells as described below:

- Aspirate the medium from the dish and add **1 mL** of freshly thawed, **room temperature** **Unlock**.
- Incubate at **37°C**, **5% CO_2** for **3-5 minutes**.
- Add **1 mL** of pre-warmed **Astrocyte Maintenance Medium** into the dish and gently re-suspend floating cell layer with a P1000 pipette.
- Transfer the cells into a 15 mL sterile conical tube containing **5 mL** pre-warmed **Astrocyte Maintenance Medium**.
- Centrifuge the cells at **400 x *g*** for **5 minutes**.
- Carefully aspirate the supernatant and gently re-suspend cell pellet in **2 mL** of **Astrocyte Maintenance Medium**.
- Remove **10 µL** of cell suspension and mix it with **10 µL** of trypan blue solution. Count the cells.
- Remove **Matrigel™** from the pre-warmed 60 mm dish and immediately transfer the **2 mL** cell solution into it. The appropriate density of cells after passaging is **80,000 – 100,000 viable cells/cm²**.
- Monitor the cell survival and attachment the following day (**Day 8**).
- On **Day 9**, replace the culture medium 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.
- The culture medium should be replaced **every 2 days** with fresh, pre-warmed **Astrocyte Maintenance Medium**.

On **Day 14**, the astrocytes growing in the 60 mm dish will be fully confluent. Coat the culture vessels required for endpoint assays one day before or on the day of passaging the cells. Passage the cells as described below:

- Aspirate the medium from the dish and add 1 mL of freshly thawed, **room temperature** **Unlock**.
- Incubate at **37°C**, **5% CO_2** for **3-5 minutes**.
- Add **2 mL** of pre-warmed **Astrocyte Maintenance Medium** into the dish and gently re-suspend floating cell layer with a P1000 pipette.
- Transfer the cells into a 15 mL sterile conical tube containing **5 mL** pre-warmed **Astrocyte Maintenance Medium**.
- Centrifuge the cells at **400 x *g*** for **5 minutes**.
- Carefully aspirate the supernatant and gently re-suspend cell pellet in **2 mL** of **Astrocyte Maintenance Medium**.
- Remove **10 µL** of cell suspension and mix it with **10 µL** of trypan blue solution. Count the cells.
- Plate the astrocytes in **Astrocyte Maintenance Medium** into the desired cell culture vessels coated with **Matrigel™** at the recommended seeding density (**80,000 – 100,000 viable cells/cm²**).
- Culture cells for an additional 2-3 days and by **Day 17**, the astrocytes can be used for experiments.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
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