

AXOL

axoCells™

**Human iPSC-Derived
Astrocytes**

User guide

Version 3

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Updates to protocol:

Part	Change	Rationale	Previous version	New version
Product ax0665 to be replaced by ax0704	New product code ax0704 added	New starting line needed (CENSOi004E)	From alternative line (NCRM5)	Includes ax0704 Astrocytes
Coating cell culture ware	Included option to use hESC-qualified Matrigel	Expands utility for users	PDL + Surebond-XF only	Includes additional reference to hESC Matrigel
Thawing Astrocytes	Changed to add cell suspension dropwise to media	Align methodology with other cell types	Media was added dropwise to cell suspension	Changed to add cell suspension dropwise to media

This protocol and its associated products are for research use only.

Products are not for diagnostic or therapeutic use and are not to be administered to humans or animals.

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Storage Once Thawed
ax0665 ax0704	axoCells Human iPSC-Derived Astrocytes	Frozen Vial, >1 x 10 ⁶ cells	N/A	-150°C / liquid nitrogen	37°C
ax0047	FGF2	100mg	N/A	-20°C	Reconstituted growth factor in 0.05% HSA can be stored in working aliquots at -20°C for up to 3 months
ax0053	Surebond-XF	1mL	200x	4°C	N/A

Additional Reagents Required		
Product Name	Supplier	Product Code
N21-Max	R&D Systems	AR008
FBS, One Shot™	ThermoFisher	A3160401
Neurobasal™-A Media		12349015
GlutaMAX™		35050061
Poly-D-Lysine (PDL)		A3890401
hESC-qualified Matrigel		354277
Heregulin	Peprotech	100-03
Human Serum Albumin (HSA)	Sigma-Aldrich	A9731

Lot-specific information such as specifications and quality control details are stated in the Certificate of Analysis. Expiry dates for Axol- supplied components are stated on the label. Consult the manufacturer's guidelines for expiry dates of any additional reagents.

IMPORTANT!

Axol Cell Culture Media DOES NOT contain antibiotics or antifungal agents. Axol 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 Media

Astrocyte Media (100mL)

Components	Volume	Stock Concentration	Final Concentration
Neurobasal™-A Medium	95mL	N/A	N/A
N21-Max	2mL	50 X	1 X
FBS, One Shot™	2mL	N/A	N/A
GlutaMAX™	1mL	100 X	1 X
Heregulin- β 1	100 μ L	10 μ g/mL	10ng/mL
FGF2	80 μ L	10 μ g/mL	8ng/mL

Prepare all above chemicals and growth factor in a class II biosafety cabinet under sterile conditions.

Preparation of 0.05% Human Serum Albumin in D-PBS

- Make a 0.05% stock concentration of HSA D-PBS by diluting 5mg HSA in 10mL D-PBS.
- Aliquot into appropriate working volumes.
- Aliquots can be stored long term at **-80°C** for up to 1 year and 2 months at **4°C**.

Preparation of Heregulin- β 1

- Make a working stock at **10 μ g/mL** by reconstituting **10 μ g** of the growth factor in 1mL HSA D-PBS.
- Aliquot into appropriate working volumes (recommended at **50 ~ 100 μ L**).
- Aliquots should be made into sterile pre-labelled tubes.
- Aliquots can be stored at **-80°C** for up to 3 months.

Preparation of FGF2

- Make a working stock at **10 μ g/mL** by reconstituting **100 μ g** of the growth factor in **10mL** HSA D-PBS.
- Aliquot into appropriate working volumes (recommended at **50 ~ 100 μ L**).
- Aliquots should be made into sterile pre-labelled tubes.
- Aliquots can be stored at **-20°C** for up to 3 months.

Coating Cell Culture Ware

A variety of extra cellular matrices can be used with the cells, i.e. PEI, Polyornithine, Poly-D-lysine, Laminin, Matrigel. We would recommend using either PDL + SureBond-XF **or** hESC-qualified Matrigel for axoCells™ astrocytes.

PDL

- Dilute the liquid poly-D-lysine 1:1 in sterile water to create a **0.05mg/mL** working solution (0.5x).
- Add **300mL per cm²** of 0.5x PDL solution (**0.05mg/mL**) to the culture vessel. Incubate for 60 mins at **37°C**, remove the solution through aspiration and thoroughly rinse the surface twice with sterile water.
- Remove water and allow the surface to dry for at least two hours before proceeding onto **SureBond-XF** coating.

SureBond-XF

- Dilute the **SureBond-XF** stock solution (200x) in D-PBS (without calcium or magnesium) to make 1x working solution, e.g., **30μL** in **6mL**.
- Coat the culture surface with **200μL per cm²** of the **SureBond-XF** 1x working solution.
- Incubate for at least 4 hours at **37°C**.
- Remove the **SureBond-XF** from the culture vessel prior to plating of cells. Do not wash the culture vessel after coating with **SureBond-XF**.
- Do not let the **SureBond-XF** coating dry out before plating the cells.

Matrigel

- Thaw hESC-qualified Matrigel overnight at **2–8°C** and keep on cold metal beads at all times during preparation and handling.
- Dilute the hESC-qualified Matrigel in cold DMEM/F-12 to the required working concentration according to the manufacturer's instructions.
- Coat the culture surface with sufficient diluted Matrigel solution to completely cover the growth area (e.g., **100μL** per well of a 96-well plate).
- Incubate the coated culture vessel for at least 45 minutes at **37°C** or overnight at **2–8°C**.
- Remove excess Matrigel solution immediately prior to cell seeding.
- Rinse residual Matrigel from the wells with PBS before seeding cells.

Thawing and Plating axoCells Human iPSC-Derived Astrocytes

1. Coat culture vessels with the substrates as described above.
2. On the day of thawing the cells prepare **Astrocyte Media** as per page 4.
3. Aliquot the media into a **50mL** sterile conical tube and pre-warm to **37°C**. Store the remaining medium at **4°C**.
4. Prepare a **15mL** sterile conical tube with **8mL** warm **Astrocyte Media**.

5. To thaw the cells, transfer the vial of cells from nitrogen storage with the vial buried in dry ice.
6. Remove the vial from dry ice and transfer it immediately to 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.
7. Do not shake the vial during thawing.
8. Spray the vial with 70% ethanol and wipe it down with a sterile paper towel before placing it in the cell culture hood.
9. Once thawed completely, use a P1000 pipette to transfer the cells into the **15mL** sterile conical tube dropwise. Gently wash the cryovial with **1mL** of the medium and transfer this to the **15mL** sterile conical tube.

IMPORTANT! Do not mix the cells vigorously. Avoid generating bubbles while pipetting.

10. Centrifuge cells at **200 x g** for **5 minutes** at room temperature.
11. Aspirate the media carefully and gently resuspend the cell pellet with **2mL** of the **Astrocyte Media** until they become mostly a single cell suspension.
12. Perform a cell count and dilute the cell suspension to the required density. We recommend a cell seeding density of **25,000 to 48,000 cells / cm²** for monoculture or **10,000 / cm²** for co-culture experiments.
13. Aspirate the spent **SureBond-XF or Matrigel** solution from the pre-coated culture vessel and then immediately transfer the cells into the culture vessel.
14. 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**).
15. Replace the culture medium on **Day 2** with fresh, pre-warmed medium. The media addition should be done gently by pointing the pipette tip toward the wall of the cell culture vessel to avoid flushing the cells directly with medium.
16. Medium changes can then be performed **every 2 days** with fresh, pre-warmed media.

Note: axoCells human iPSC-derived astrocytes are **assay-ready day 2 post thaw**. Prolonged culture of these cells is not necessary for functional maturation.

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