axoCells™
Human iPSC-Derived Microglia
User Guide

Version 5

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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.

Updates to protocol

Part	Change	Rationale	Previous version	New version
Coating Cell Culture Ware	Use PBS to wash coating off plates instead of sterile water	Better adherence of cells	Use water to wash	Use PBS (-/-) to wash
Thawing axoCells Human iPSC-Derived Microglia	ROCKi (Y-27632) used at thaw instead of Thiazovivin	Better thaw recovery	Thiazovivin used at 10 mM	y-27632 used at 10 mM
	Changed the wording around thaw process	Emphasize process is done gently and quickly		
Plating axoCells Human iPSC-Derived Microglia	Removed 20 min wait between plating cells and putting in incubator	No need for this step. Does not add value	Leave plates in the hood after seeding cells for 20min	Place plates with cells in the incubator directly after thaw
Preparation of Reagents	Addition of 2-mercaptoethanol to Complete Microglia Media	2-mercaptoethanol is unstable in solution so should be added immediately before use	2-mercaptoethanol was previously included in ax0660d but removed due to unstable nature	Add 5µM 2-mercaptoethanol to Complete Microglia Media ***NOTE: only applies to media received**

				Aug2025 or after*

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0664	axoCells Human iPSC-Derived Microglia (Male)	Frozen Vial, 1 x 10^6cells	N/A	-150°C / liquid nitrogen	See Protocol	37°C
ax0660	Microglia Maintenance Media + Supplement A, B and C	100 ml + 100 µl +100µl + 1 ml	1x	Store Microglia Maintenance Media at 4°C Store supplements A, B and C at -80°C	Supplement A at Room Temperature Supplement B at Room Temperature Supplement C at 4°C	Use immediately or store for 7 days at 4°C
ax0053	Surebond-XF	1ml	100x	4°C	Store at 4°C until date of expiry.	N/A

Additional Reagents Required			
Product Name	**Supplier**	**Supplier Product Code**	**Storage on Arrival**
Concanavalin A	Sigma	C2010 - 25MG	-20°C
Y-27632	Focus Biomolecules	72304-5mg	-20°C
PBS (-/-)	Thermo Fisher	10010023	RT
2-mercaptoethanol	Thermo Fisher	31350010	4°C

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 Reagents

Microglia Maintenance Media

- Upon receipt, aliquot and store un-supplemented **Microglia Maintenance Media** at 4°C.

- **Microglia Maintenance Media** requires supplementing with Supplement A, B, C and 2-mercaptoethanol before use**.

NOTE: 2-mercaptoethanol should only be added to media received in or after August 2025 where the label specifies this addition is needed. Does not apply to Lots: 0660200325d, 0660060625d and 0660150725d

- Add the Supplements and 2-mercaptoethanol to **50ml of Microglia Maintenance Media** according to the table below to create **Complete Microglia Media**. Once fully-supplemented the **Complete Microglia Media** is stable for 1 week at 4°C.

Supplement	Final Concentration	50 ml Microglia Maintenance Media
Supplement A	10 ng/ml	50 µl
Supplement B	100 ng/ml	50 µl
Supplement C	1 x	500 µl
2-mercaptoethanol (50mM)	5µM	5 µl

- Before use, pre-warm an aliquot of **Complete Microglia Media** to 37°C.

Supplement A

- Store **Supplement A** at -80°C on arrival.
- Thaw at room temperature and use 50 µl of **Supplement A** to make up 50 ml of **Complete**
- **Microglia Media**. Aliquot the remaining 50 µl and store at -80°C until required. Do not thaw and refreeze more than once.

Supplement B

- Store **Supplement B** at -80°C on arrival.
- Thaw at room temperature and use 50 µl of **Supplement B** to make up 50 ml of **Complete Microglia Media**. Aliquot the remaining 50 µl and store at -80°C until required. Do not thaw and refreeze more than once.

Supplement C

- Store **Supplement C** at -80°C on arrival.
- Thaw at 4°C. Use 500 µl of **Supplement C** to make up 50 ml of **Complete Microglia Media**. Aliquot the remaining 500 µl and store at -80°C until required. Do not thaw and refreeze more than once.

Y-27632

- Upon receipt, store **Y-27632** at -20°C.
- Prepare aliquots of **Y-27632** by resuspending in 1.56ml of sterile water to get a stock concentration of 10mM.

Concanavalin A

- Upon receipt, store **Concanavalin A** at -20°C
- Prepare aliquots of **Concanavalin A** by adding 25 ml sterile D-PBS to 25mg of powder for a 1 mg/ml solution.
- Filter the solution through a 0.22µm PES filter, make 1 ml aliquots and store at -20°C for up to 3 months.

Coating Cell Culture Ware

- axoCells microglia require both **Concanavalin A** and **Surebond-XF** in order to attach to cell culture surfaces. The same coating procedure below can be used for both plastic and glass. Microglia may be thawed directly onto cell culture treated plastic, for example when cells need to be re-plated at a later date. However, this may result in some cell loss and Axol have not performed quality control using this methodology.
- Thaw an aliquot of **Concanavalin A.**
- Add **Surebond-XF** and **Concanavalin A** in a 1:100 dilution in sterile D-PBS to give a final concentration of 5 µg/ml **Surebond-XF** and 10 µg/ml **Concanavalin A**.
- Add to cultureware at 312 µl per cm^2 of growth area.
- Incubate coating mixture for at least 4 hrs or overnight at 37°C, 5% CO_2.
- Aspirate coating and wash thoroughly **three times** with PBS (-/-) in a volume equal to the coating volume.
- Leave the culture vessel open in a biological safety cabinet until surface is **completely dry.** This can take anywhere between 45 minutes to 3 hours depending on the vessel used.
- Coated cultureware should preferably be used the same day, though can be kept for 7 days at 4°C without appreciable loss of attachment function.

Thawing axoCells Human iPSC-Derived Microglia

- Prepare **Microglia Plating Media**. For this, thaw an aliquot of **Y-27632** and add 1:1000 to **Complete Microglia Media** to give a final concentration of 10µM **Y-27632**.
- The thawing process should be performed as quickly as possible to avoid excessive cell death. While cells are in cryopreservation media, they are more fragile.
- Prepare a 15ml conical tube by adding 10 ml of **Complete Microglia Media**.
- Remove the vial from dry ice and transfer immediately to a 37ºC water bath. Do not completely submerge the vial (only up to 2/3 of the vial). Remove the vial before the last bit of ice has melted.
- Do not shake the vial during thawing.
- Spray the vial with 70% ethanol and wipe it down with a sterile paper towel before placing it in the cell culture hood.
- Transfer the cells gently into the prepared 15ml conical tube containing medium doing this **dropwise and slowly**, whilst gently moving the tube back and forth. This reduces the effects of osmotic shock and maximizes cell viability.
- Rinse the vial with 1 ml pre-warmed 37°C **Complete Microglia Media** and combine dropwise and slowly with the cell suspension in the 15 ml conical tube.
- Centrifuge the cells at **300 x g for 5 minutes**.
- Discard most of the supernatant by aspiration and use a P200 pipette to remove the last remnants of supernatant. Be careful not to disturb the pellet.
- Re-suspend the cell pellet in 1 ml pre-warmed, 37°C **Microglia Plating Media** (Complete Microglia Media + 10 µM Y-27632).
- Pipette the cells up and down several times with moderate force to achieve a homogeneous single cell suspension.
- Take the average of three cell counts to determine the total number of viable cells in the vial.

Plating axoCells Human iPSC-Derived Microglia

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number per well	Plating Density (cells per ml)
6-well plate	10	2	1×10^6	500,000
12-well plate	4	1	400,000	400,000
24-well plate	2	0.5	200,000	400,000

96-well plate	0.33	0.1	33,000	330,000

- The recommended seeding density for axoCells microglia is **100,000 cells/cm²**. Cells should not be seeded below this density but can be seeded higher if desired. The table below summarizes recommended volumes and plating densities for microglia seeded at the recommended 100,000 cells/cm². All measures are *per well.*
- axoCells microglia can be seeded at densities ranging from 100,000 - 300,000cells/cm². The user, however, should determine the appropriate seeding density for their application empirically.
- Once the total number of viable cells has been determined, dilute the cell suspension in **Microglia Plating Media** to obtain the desired platingdensity.
- Mix the cell suspension either by inversion of the tube or with an electronic pipette.
- Quickly, add the cell suspension to the coated cell culture vessel and immediately agitate the vessel to disperse the cells evenly across thesurface.
- Incubate at 37°C, 5% CO_2.

Maintenance of axoCells Human iPSC-Derived Microglia

- Pre-warm an aliquot of **Complete Microglia Media** to 37°C. Y-27632 is only required for the **Microglia Plating Media**.
- The day after seeding, perform a **full media change** with **Complete Microglia Media** (no Y-27632). Perform a half media change **every 2-3 days** thereafter.
- Allow cells to recover for at least **7 days** before performing assays or experiments.

Day Post-thaw	Action(s)		Information
0	Make up 50ml Microglia Maintenance Media	Plate Cells	
1	Remove & Replace **100%** of the Media		
2			
3	Remove & Replace 50% of the Media		
4			
5	Remove & Replace 50% of the Media		
6			
7	Remove & Replace 50% of the Media		Ready for Experiments
8			
9	Remove & Replace 50% of the Media		
10			
11	Remove & Replace 50% of the Media		
12			
13	Remove & Replace 50% of the Media		
14			

Got any questions? Need help with the protocol?

Contact Axol Technical Support

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Or

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