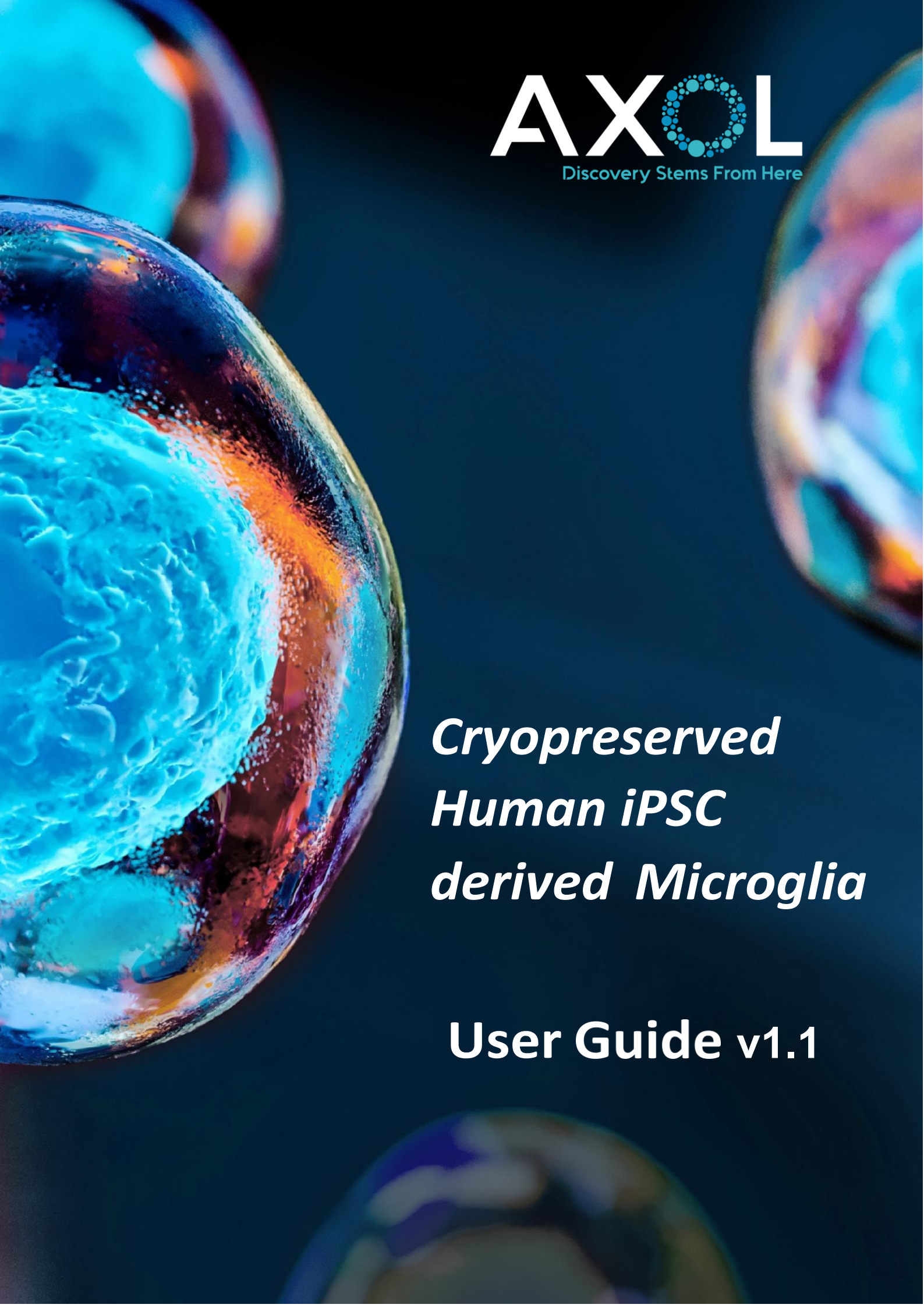

Cryopreserved Human iPSC derived Microglia

User Guide v1.1

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Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0668	Human iPSC-Derived Microglia (Female)	Frozen Vial, 1 x 10^6 cells	N/A	-196°C	See Protocol	37°C
ax0660	Microglia Maintenance Medium + Supplement A, B and C	100 mL + 100 µL + 100 µL + 1 mL	1x	Store Microglia Maintenance Medium at 4°C Store supplements A, B and C at -80°C	Supplement A – RT Supplement B – RT Supplement C – 4°C	Use immediately and re-aliquot remaining for storage at -80°C
ax0053	Surebond-XF	1mL	200x	4°C	N/A	N/A

Additional Reagents Required			
Product Name	**Supplier**	**Supplier Product Code**	**Storage on Arrival**
Concanavalin A	Sigma	C2010-25MG	-20°C
Thiazovivin	Focus Biomolecules	10-1191 – 5MG	-80°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 Bioscience 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.

Recommendations

- Recommended cell culture medium: Microglia Maintenance Medium supplemented with Supplement A, B and C

Preparation of Reagents:

Microglia Maintenance Medium

Supplement A

- Store Supplement A at -80ºC on arrival.
- Thaw at room temperature and use 50 µL of Supplement A to make up 50 mL of Complete Microglia Maintenance Medium. Aliquot the remaining 50 µL and store at -80ºC until required. Do not thaw and refreeze more than once.

Supplement B

- Store Supplement B at -80ºC on arrival.
- Thaw at room temperature and, use 50 µL of Supplement B to make up 50 mL of Complete Microglia Maintenance Medium. Aliquot the remaining 50 µL and store at -80ºC until required. Do not thaw and refreeze more than once.

Supplement C

- Store Supplement B at -80ºC on arrival.
- Thaw at 4ºC. Use 500 µL of Supplement C to make up 50 mL of Complete Microglia Maintenance Medium. Aliquot the remaining 500 µL and store at -80ºC until required. Do not thaw and refreeze more than once.

Making up Complete Medium

- Upon receipt, aliquot and store Microglia Maintenance Medium at 4ºC.
- Microglia Maintenance Medium requires supplementing with Supplement A, B and C before use.
- Add the Supplements to the Microglia Maintenance Medium according to the table.
- Complete, fully supplemented, Microglia Maintenance Medium is stable for 1 week at 4ºC.
- Before use, pre-warm an aliquot of Microglia Maintenance Medium to 37ºC.

Supplement	Final Concentration	50 mL Microglia Maintenance Medium
Supplement A	10 ng/mL	50 µL
Supplement B	100 ng/mL	50 µL
Supplement C	1 x	500 µL

Thiazovivin

- Upon receipt, store Thiazovivin vial at -80°C
- Prepare a 10mM stock of Thiazovivin by dissolving 5mg powder in 1606 µL of DMSO.
- Make 50 µL aliquots and store at -80°C for up to 2 months
- Use freshly each time and do not re-freeze

Concanavalin A

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

Coating Cell Culture ware

Axol's cryopreserved microglia require both Concanavalin A and Surebond-XF in order to attach to cell culture surfaces. They are not amenable to other coating substrates.

The same coating procedure below can be used for both plastic and glass.

- Thaw an aliquot of Concanavalin A
- Add Surebond-XF and Concanavalin A in a 1:100 dilution in sterile DPBS to give a final concentration of 5 μg/mL Surebond-XF and 10 μg/mL Concanavalin A
- Add to culture ware at 1mL per 10cm^2 growth area.
- Incubate coating mixture for at least 4hrs or overnight at 37°C, 5% $C0_2$
- Aspirate coating and wash thoroughly **three times** with sterile distilled water 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 culture ware should preferably be used the same day, though can be kept overnight at 4°C without appreciable loss of attachment function

Thawing Human iPSC-derived Microglia

- Prepare **Microglia Plating Medium**. For this, thaw an aliquot of Thiazovivin and add 1:1000 to Complete Microglia Medium to give a final concentration of 10μM Thiazovivin
- Remove the vial of frozen microglia from liquid nitrogen storage
- Ensure the cap is tight. Roll the vial between your gloved hands until the outside is free of frost.
- Immerse the vial in a 37°C water bath until about ¾ of the way down, without submerging the cap. Swirl the vial gently. When only a pea-sized ice crystal remains, remove the vial from the water bath
- Spray with vial with 70% ethanol, wipe dry and bring the vial into the biological safety cabinet
- Transfer the cells gently into a sterile 50 ml conical tube using a 1 ml pipette
- Rinse the vial with 1 ml pre-warmed 37°C Complete Microglia Medium and combine **dropwise and slowly** with the cell suspension in the 50 ml conical tube, whilst gently moving the tube back and forth. This reduces the effects of osmotic shock and maximizes cell viability.
- Slowly, using a minimal speed setting on an electronic pipette, add 8 ml of warmed, 37°C Complete Microglia Medium to the cells in a drop-wise fashion whilst swirling the tube. This should be done over a 30 second period.
- Centrifuge the cells at **300 *x g* for 10 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, 37ºC Microglia Plating Medium** (Complete Microglia Medium + 10uM Thiazovivin).
- Pipette the cells up and down several times with moderate force to achieve a homogenous single cell suspension
- Take the average of three cell counts to determine the total number of viable cells in the vial

Plating Human iPSC-derived Microglia

The recommended seeding density for Human iPSC-derived Microglia is 100,000 cells/cm^2. Cells should not be seeded below this density but can be seeded higher is desired.

- Once the total number of viable cells has been determined, dilute the cell suspension in Microglia Plating Medium to obtain the desired plating density
- 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 the surface
- Leave undisturbed for 20 minutes on an even surface in a biological safety cabinet to allow the cells to attach
- Incubate at 37°C, 5% $C0_2$

Human iPSC-derived Microglia can be seeded at densities ranging from 100,000 - 300,000 cells/cm^2. The user however should determine the appropriate seeding density for their application empirically.

The table summarises recommended volumes and plating densities for microglia seeded at the recommended 100,000 cells/cm^2. All measures are *per well.*

Culture Vessel	Surface Area (cm^2)	Plating Volume (mL)	Cell Number to Seed (100K/cm^2)	Plating Density (cells per mL)
6-well plate	10	2	1 x 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	0.2	30,000	150,000

Maintenance of Human iPSC-derived Microglia

- Pre-warm an aliquot of **Complete Microglia Medium** to 37oC
- The day after seeding, perform a **full media change** with Complete Microglia Medium
- Perform a half medium change **every 2 days** thereafter
- Allow cells to recover for at least **5 days** before performing assays or experiments

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

Got any questions? Need help with the User Guide?
Contact Axol Technical Support at
support@axolbio.com
Or
Call +44 (0) 1223 751051 (UK)
+1-800-678-AXOL (2965) (US Toll-free)