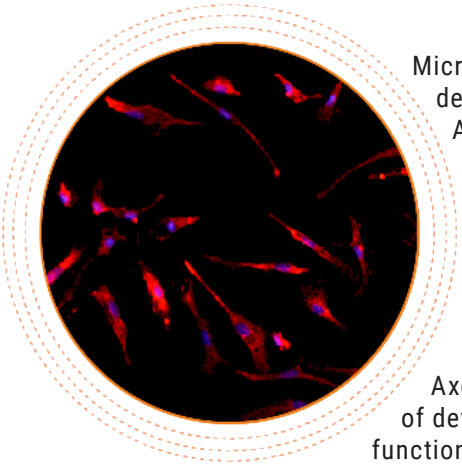
Human iPSC-Derived Microglia



Microglia are the immune cells of the central nervous system, with key roles in brain development, neurogenesis, synaptic pruning and homeostatic maintenance. As inflammation is linked to many neurological diseases such as Alzheimer's, Parkinson's, ALS and aging, microglia are becoming targets for treatments of these conditions.

In the brain, microglia are highly dynamic, moving constantly to actively survey the brain parenchyma. There is abundant evidence that activated microglia can be harmful to neurons.

Axol's method for generating iPSC-derived microglia mimics the *in vivo* pathway of development for brain resident macrophages and produces microglia that are functionally representative of primary human microglia *in vitro*.

Human iPSC-Derived Microglia

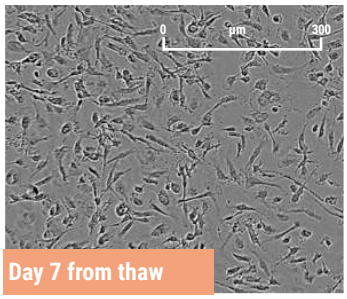
PRODUCT CODE	PRODUCT NAME	DONOR	STARTING MATERIAL	QUANTITY
ax0664	Human iPSC-Derived Microglia: Cryopreserved	Male, age 40-50 years	Fibroblasts cells	1 million cells per vial
ax0679	Microglia Complete Cells and Media			1 vial of ax0664 1 vial of ax0660

Microglia Culture Media and Reagents

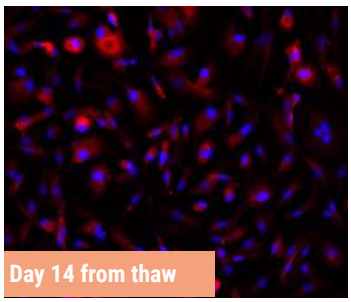
PRODUCT CODE	PRODUCT NAME	QUANTITY
ax0660	Microglia Maintenance Medium Kit	100 mL

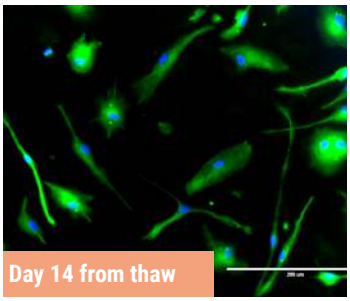
Functional Characterization of Microglia

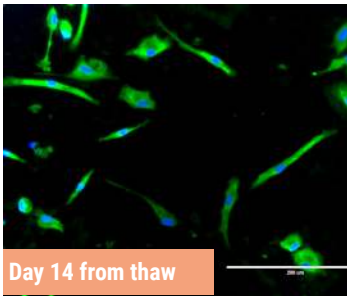
Human iPSC-derived microglia were matured for 7 days prior to cryopreservation. Cells were then thawed onto 96-well plates coated with Surebond-XF and Concanavalin A at a density of 100,000 cells/cm^2 in Axol Microglia Maintenance Medium. Microglia adhere strongly to the matrix and display a ramified morphology and are assay ready after 7 days.



Phase contrast image of microglia 7 days post thaw, cultured in Axol Maintenance Media. Cells seeded at 100,000 cells/cm^2





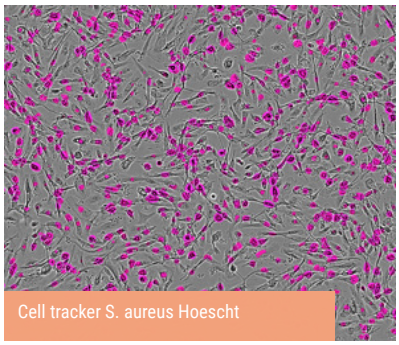


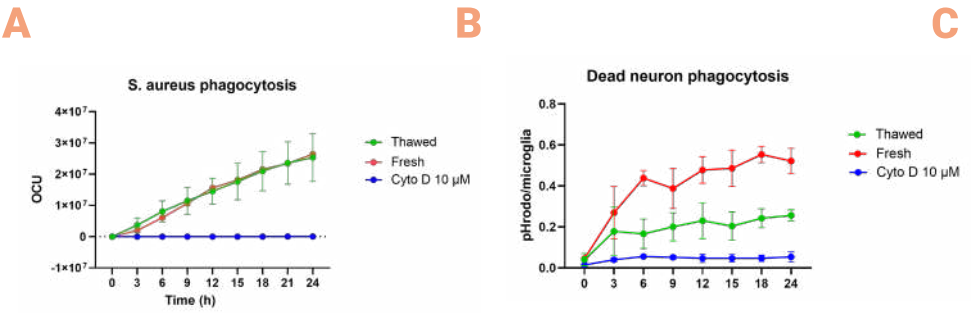
Microglia 14 days post thaw, cultured in Axol Maintenance Media. Cells seeded at 100,000 cells/cm^2, x20 magnification. Markers: A) Red: P2RY12, Blue: DAPI, B) Blue: DAPI, Green: IBA-1, C) Blue: DAPI, Green: TMEM119, D) Blue: DAPI, Green: CXCR1

Axol's iPSC-Derived Microglia show dynamic surveillance behavior, which is representative of their phenotype *in vivo*.

Microglia Phagocytosis Assay

Mature human iPSC-derived microglia are functionally active. This is best shown by a phagocytosis assay using pHrodo labeled bait such as bacteria, dead neurons and more, which fluoresce when engulfed in the acidic environment of a microglial phagosome. **Custom labeling of baits are available.**



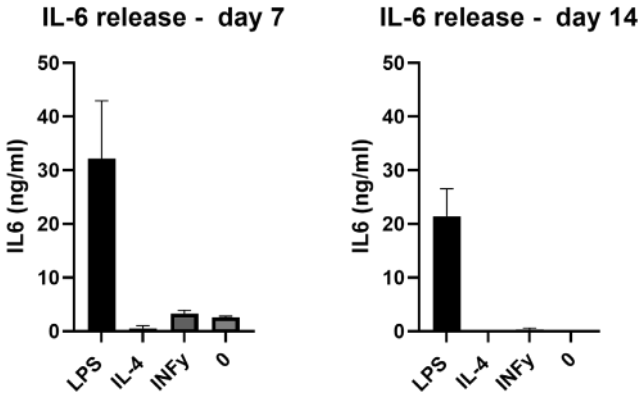


Phagocytosis assay was performed by incubating iPSC-derived microglia with pHrodo labelled S. aureus or dead neurons. Phagocytosis was measured over time using an IncuCyte® S3. A) Representative image of phagocytosis masking using the IncuCyte. Uptake of B) S. aureus and C) dead neurons over 24h was compared between thawed and fresh cells. Cytochalasin D (10uM) was used as a negative control. -

Cytokine Release

Additional functional readouts for cryopreserved microglia includes IL-6 cytokine release following stimulation. Activation was assessed 7 and 14 days post thaw.

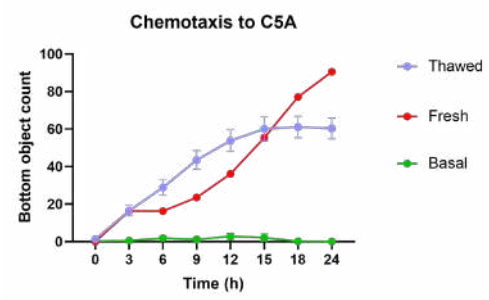
Cytokine release following 24h stimulation with either Lipopolysaccharide (LPS), IL-4 or interferon gamma (INFy). Microglia were thawed and cultured for 7 or 14 days prior to stimulation and supernatant collected. IL-6 was measured using an HTRF assay.



Chemotaxis Assay

Chemotaxis of microglia can be assessed using the IncuCyte S3. Cryopreserved microglia migrate to Complement C5A in a similar manner to fresh microglia over a 24h timecourse.

Chemotaxis to C5A measure using the IncuCyte S3. Chemotaxis of fresh and cryopreserved microglia was assessed over 24h. Bottom object count was used as a masure of migration.



Conclusion

Axol Bioscience human iPSC-derived microglia are manufactured in our ISO 9001:2015 qualified labs and demonstrate structural and functional properties to enable modelling of neurodegenerative disease *in vitro*.