Optimization of the Seahorse XF Assay for iCell® Microglia

iCell Lab Note

Introduction.

Microglia are the resident immune cells in the CNS and are key players in neurogenerative and neuroinflammatory diseases. There is an extreme amount of diversity within microglia, not only functionally and morphologically, but also with respect to their metabolic versatility. Knowledge of the cellular metabolism of apparently healthy normal (AHN) human iPSC-derived microglia as well as microglia with Alzheimer's disease-relevant mutations (i.e., TREM2 or ApoE) may provide new treatment avenues for therapeutic drug discovery research. Assays that provide a foundational understanding of the baseline metabolic activity must first be established. This document provides a technical overview and example data for performing bioenergetic measurements with iCell® Microglia in 96-well format using the Seahorse XF Pro Analyzer from Agilent Technologies.

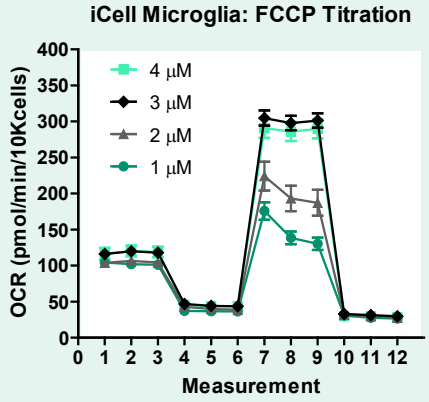


Figure 1. Assay optimization data. iCell Microglia were plated at a density of 30K microglia per well and the XF Cell Mito Stress Kit was run on Day 3. A range of FCCP concentrations were tested, with 3 µM providing the most optimal results (highest respiration).

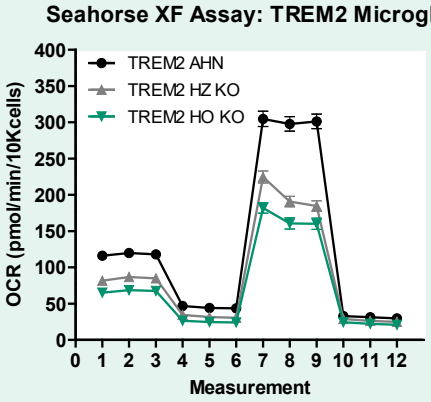


Figure 2. Disease Modeling. Mitochondrial function of iCell Microglia was compared to other microglia with Alzheimer's disease-relevant TREM2 mutations. The data suggest that differences at the genetic level can drive functional phenotypic differences at the cellular level, specifically a decrease in maximum respiration.

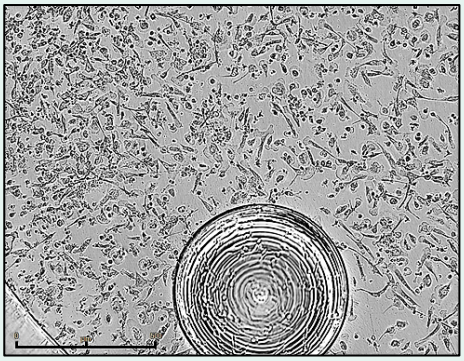
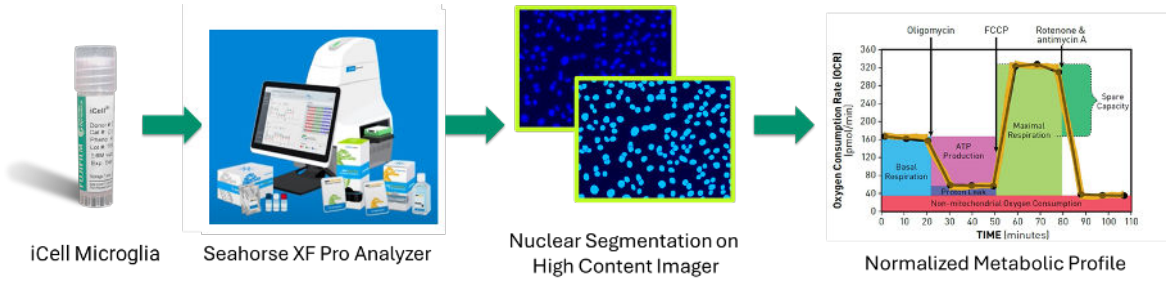


Figure 3. Microglia morphology. Visualizing the cells prior to assay is very helpful as microglia can display varied structural features depending on cell culture surface, ECM, or media formulation. Cells pictured here are happy, healthy, and represent the typical morphology and density plated for iCell Microglia in this assay. Scale bar represents 500 µm.

Assay Workflow Schematic.



iCell Microglia | Seahorse XF Pro Analyzer | Nuclear Segmentation on High Content Imager | Normalized Metabolic Profile

Methods.

Prepare iCell Microglia Complete Maintenance Medium and thaw according to the iCell Microglia Quick Guide.

- On Day 0, thaw iCell Microglia and add cells (80 µl/well) to a 96-well PDL-coated plate at a density of 30,000 cells/well.
- Incubate iCell Microglia for 3 days after plating.
- On Day 2, hydrate XF Sensor Cartridge overnight.
- On Day 3, exchange spent microglia medium with 180 µl of XF Assay Medium.*
- Run Seahorse XF Assay** as recommended by Agilent: https://www.agilent.com/en/product/cell-analysis/how-to-run-an-assay
- Label cells with nuclear dye in XF Assay Media (20 µl of 1:1000 working solution to each well for 15 min).
- Acquire fluorescent images on microscope or high content imager; perform nuclear segmentation; export Excel data.
- Copy/paste cell number data to the "Normalize" tab in results page of XF Pro Controller Software.
- Alternatively, upload data to cloud-based Seahorse Analytics: https://seahorseanalytics.agilent.com/Account/Login

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell Microglia Kit, 01279	FCDI	R1131
▪ iCell Microglia, 01279	(incl. in kit)	C1110
▪ iCell Glial Base Medium	(incl. in kit)	M1054
▪ iCell Microglia Supplement A	(incl. in kit)	M1036
▪ iCell Microglia Supplement B	(incl. in kit)	M1037
▪ iCell Neural Supplement C	(incl. in kit)	M1055
XF Cell Mito Stress Test Kit	Agilent	103015-100
XF Pro M FluxPak mini †	Agilent	103777-100
XF Pro PDL Cell Culture Microplate ‡	Agilent	103799-100
XF DMEM Assay Medium Pack	Agilent	103680-100
Hoecht 33342 Nucleic Acid Stain	ThermoFisher	H3570
Seahorse XF Pro Analyzer	Agilent	S7850A

† FluxPak includes required sensor cartridges and calibrant solution.
‡ Manual coating of an XF Pro M Cell Culture microplate (1103774-100) with GIBCO™ Poly-D-Lysine solution (A3890401) is also acceptable.

*** Recommended XF Assay Medium** for iCell Microglia is Seahorse XF DMEM Medium, pH 7.4 (which contains HEPES) that is further supplemented with 10 mM glucose, 1 mM Sodium Pyruvate, and 2 mM L-Glutamine. **** Optimized Compound Concentrations** are 1 µM oligomycin, 3 µM FCCP, and 0.5 µM Rotenone/Antimycin A.

Summary.

This lab note provides a brief overview and optimized conditions (including cell density, [FCCP], etc.) for running a Seahorse XF Assay with iCell Microglia. The assay can be used to investigate differences in TREM2 variants (Cat. #C1134 for TREM2 HZ KO and #C1136 for TREM2 HO KO), other products from the iCell Microglia Alzheimer's Disease (AD) portfolio, or across pro- or anti-inflammatory stimulation conditions. Additionally, these protocol guidelines can be applied to different Agilent kits, such as the XF Real-Time ATP Rate Assay or the XF Substrate Oxidation Stress Test to gain deeper insights into the glycolytic activity of microglia as well as changes in the metabolic flexibility of these cells.

Highlights.

Critical parameters for using iCell Microglia on the Seahorse XF Pro Analyzer have been optimized so you can easily implement the assay.

TREM2 mutations impact the bioenergetic profile of iCell Microglia, suggesting relevance for AD research.

Additional assay kits are available to further dissect the metabolic flexibility of iPSC-derived microglia.

Scan here to download the iCell Microglia Quick Guide.

Contact **Technical Support** for more protocol details and supportive data.
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LN-X4004_0125