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Value from Innovation

Cellular Dynamics

Performing a Seahorse XF Assay with iCell® Macrophages 2.0

iCell Lab Note

Introduction.

Macrophages are phagocytic cells of the innate immune system that are involved in degradation of cellular debris and pathogens, antigen presentation, and cytokine or chemokine release. The metabolic program controlling macrophage activation and function is an emerging area in studying the immune response. The regulation of immunometabolism can be assessed to better understand human macrophage function. This iCell® Lab Note provides a technical overview for and representative data using iCell Macrophages 2.0 in 96-well plates for bioenergetic measurements on the Seahorse XF Pro platform from Agilent Technologies.

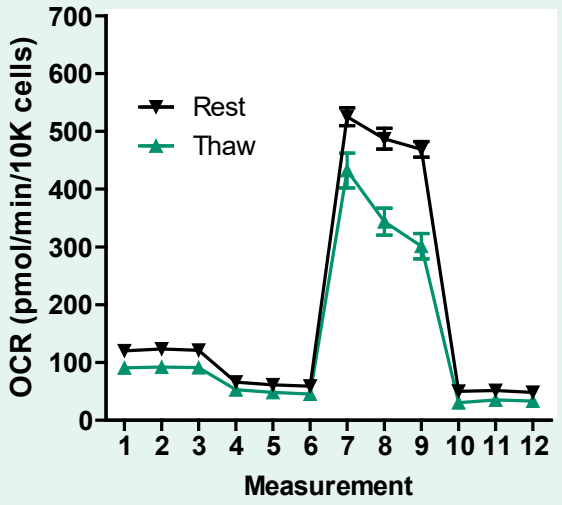


Figure 1. Post-thaw Assay Timing Comparison. iCell Macrophages 2.0 were assayed either immediately post-thaw (DIV 0) or after allowing the cells to rest and recover after plating (DIV 3). 30K cells per well were seeded on PDL-coated assay plates. Incorporating a rest period into the assay workflow is recommended based on signal consistency from measurements 7-9 (2 µM FCCP treatment for Maximal Respiration).

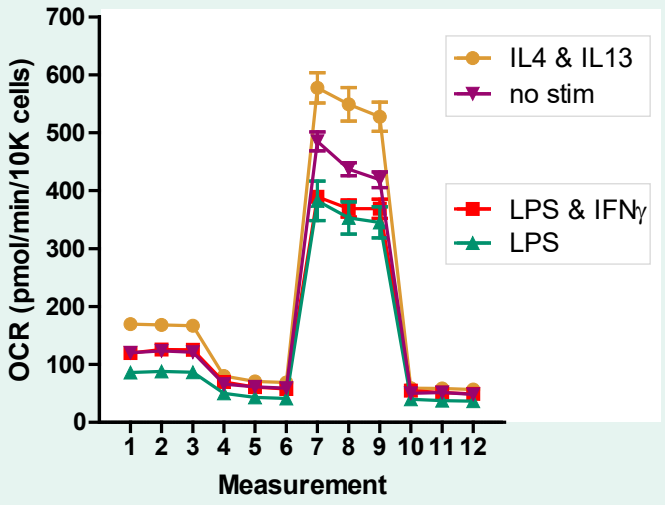


Figure 2. Macrophage Polarization. Seahorse XF assay was performed with 30K iCell Macrophages 2.0 per well on DIV 3 that were treated with pro-inflammatory (100 ng/ml LPS ± 50 ng/ml IFNγ) or anti-inflammatory stimuli (50 ng/ml IL-4 and IL-13) for 24 h. The OCR signal and metabolic profile was lower for pro/M1 stimulation compared to an untreated control but was enhanced over the naïve state when anti/M2 stimuli were used.

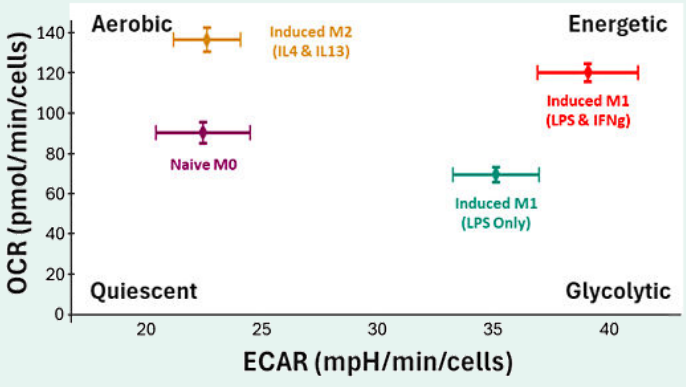
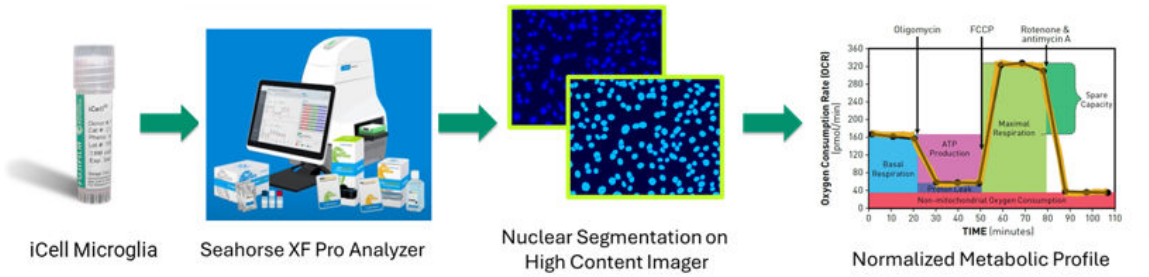


Figure 3. Metabolic Classification. OCR vs. ECAR plot from the Mito Stress Test data indicating that the M1 triggered response of iCell Macrophages 2.0 to pathogenic stimuli (LPS and IFNγ) is a glycolytic phenotype whereas the cellular response to M2 stimuli (IL-4 and IL-13) appears to be more aerobic and dependent on oxidative phosphorylation for their metabolism. This type of data analysis can be extracted from different XF assays available to evaluate the bioenergetic immune response of human iPSC-derived macrophages.

Assay Workflow Schematic.



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

Methods.

Prepare the Macrophage Thawing and Macrophage Maintenance Media and thaw cells according to User's Guide.

- Day 0, add 30,000 cells in 80 µl/well to a 96-well PDL-coated plate (excluding 4 corners).
  - Incubate cells for up to 3 days post-plating.
- Day 2, hydrate XF Sensor Cartridge overnight.
  - If desired, stimulate cells overnight at this point.
- Day 3, carefully exchange Macrophage Maintenance Medium with XF Assay Medium for final volume of 180 µl.*
  - Using smaller volumes (e.g., 100 µl) two times is more thorough and will not disturb the cells as much.
- Run Seahorse XF Assay as recommended by Agilent.
  - Optimized [compounds] are listed to the right.**
  - Refer to guidelines and instructions here: https://www.agilent.com/en/product/cell-analysis/how-to-run-an-assay
- When the assay is complete, label cells with 20 µl of Hoechst nuclear stain for 15 min.
- Acquire images on fluorescent microscope or high content imager; perform cell segmentation and counts; export data to Excel.
- 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 Macrophages Kit, 01279	FCDI	R1186
▪ IMDM	Thermo Fisher	12440-053
▪ GlutaMAX Supplement	Gibco	35050-061
▪ SFEM	STEMCELL Tech	09650
▪ Pen/Strep (optional)	Gibco	15140
LPS from *E. coli* O127:B8 ◊	Sigma	L4516
XF Cell Mito Stress Test Kit	Agilent	103015-100
XF Pro M FluxPak mini †	Agilent	103777-100
PDL Cell Culture Microplate ‡	Agilent	103799-100
XF DMEM Assay Medium Pack	Agilent	103680-100
Hoechst 33342 Nuclear Stain	Thermo Fisher	H3570
Seahorse XF Pro Analyzer	Agilent	S7850A

◊ All other stimuli were from PeproTech / Thermo Fisher.
† 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) has also been tested.
*** Recommended XF Assay Medium** is Seahorse XF DMEM Medium, pH 7.4 (w/ HEPES), further supplemented w/ 10 mM glucose, 1 mM Sodium Pyruvate, and 2 mM L-Glutamine.
** Oligomycin (1 µM), FCCP (2 µM), and Rotenone/Antimycin A (0.5 µM).

Summary.

This iCell Lab Note establishes the starting experimental conditions for running a Seahorse XF Assay with iCell Macrophages 2.0. The robust metabolic measurements acquired from these cells suggest that these guidelines can be applied to other Seahorse XF assays, such as the XF Real-Time ATP Rate Assay for quantitative assessment of glycolytic activity and total ATP production to gain deeper insight on bioenergetic activity of macrophages or the XF substrate oxidation assay for comprehensive characterization of full dependencies and the metabolic flexibility of these cells.

Highlights.

Essential test parameters have been optimized so you can start an XF Assay with iCell Macrophages 2.0 right away.

Seahorse XF technology can be used to monitor immune regulation and the M1/M2 polarization of macrophages.

iCell Macrophages 2.0 are reliable source of human cells that are ready-to-use for numerous applications.

Scan here to download the iCell Macrophages 2.0 User's Guide.

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