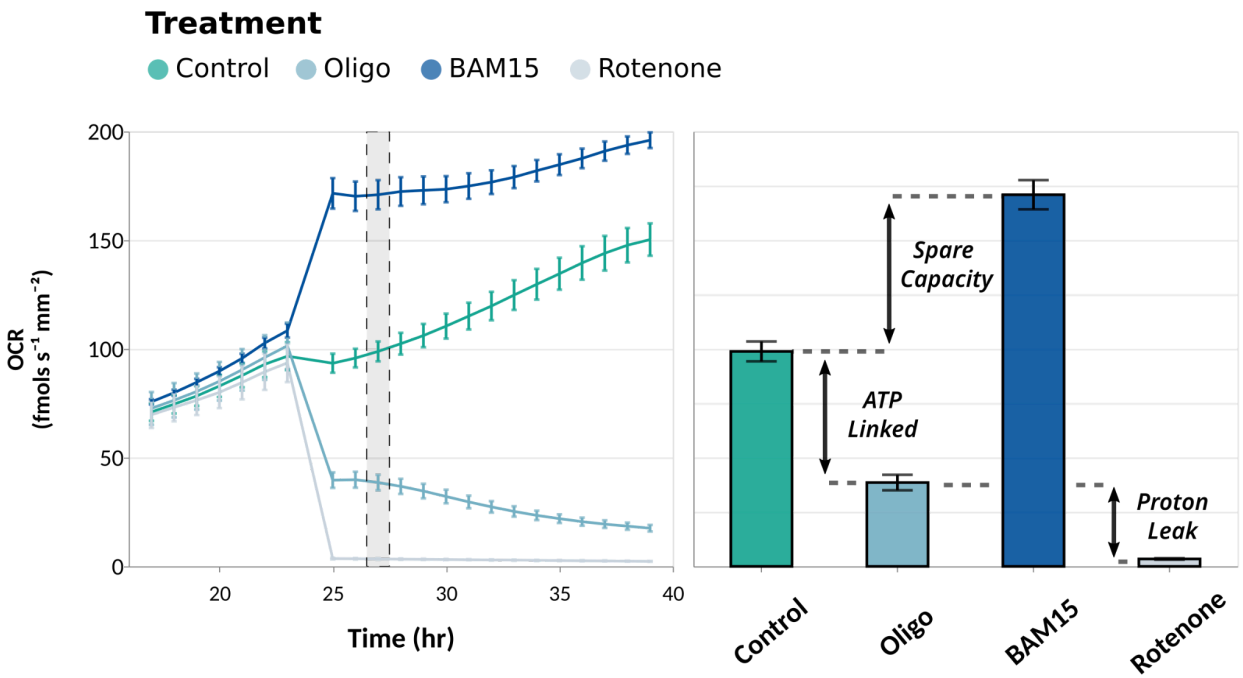
Lucid Scientific

Resipher Bioenergetic Profiling Guide

Overview

The Resipher's continuous OCR readout enables bioenergetic profiling of mitochondrial function. This document outlines a generalized protocol for assessing mitochondrial function using Electron Transport Chain (ETC) modulating treatments (e.g., Oligomycin, BAM15 or FCCP if needed, Rotenone/AA etc.).

Introduction



Understanding metabolism and energy production within cells and tissues is essential for studying biological functions, disease mechanisms, and potential treatments. This critical understanding can be achieved through bioenergetic profiling with Resipher.

Resipher provides non-invasive, long-term monitoring of cellular Oxygen Consumption Rate (OCR), an ideal readout for characterizing mitochondrial function. This protocol outlines the application of Resipher, providing a step-by-step guide along with best practices and common pitfalls to ensure accurate and reproducible results.

Protocol Summary

Seed cells in standard well plates, using established protocols and allow the cells to settle / adhere. Replace the well plate lid with Resipher and establish a culture baseline while receiving real-time growth insights with Resipher.

When running a bioenergetic profile it is important to ensure that you have a baseline measurement of OCR for each experimental group. This baseline should be performed for a duration of 8-24 hours to acquire an accurate pre-treatment baseline. To ensure the full range of response to treatment is visualized, baseline OCR ideally falls within 30–100 fmols/min/mm^2.

Apply ETC modulators using a media volume small enough to prevent diffusion-limited effects (typically a 70 µL media volume) to reliably capture OCR changes and ensure accurate response detection.

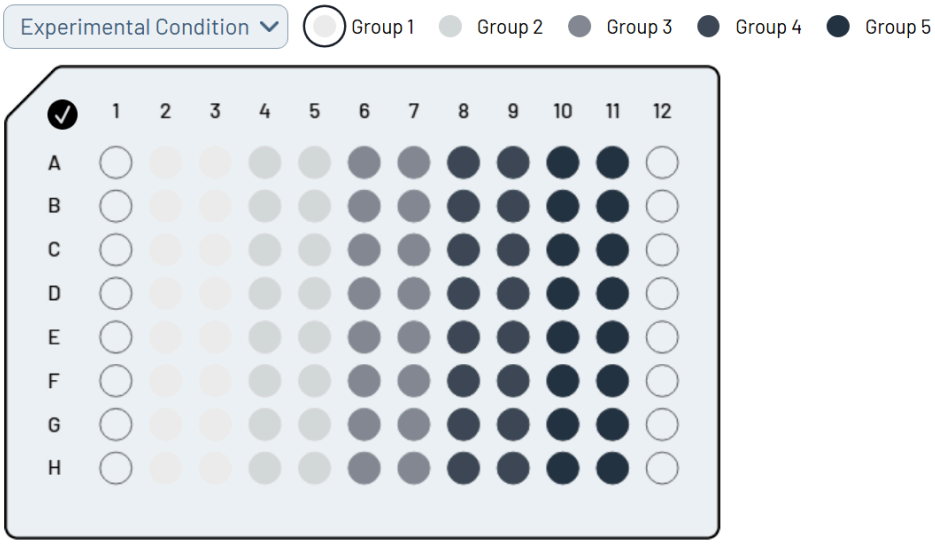
Materials

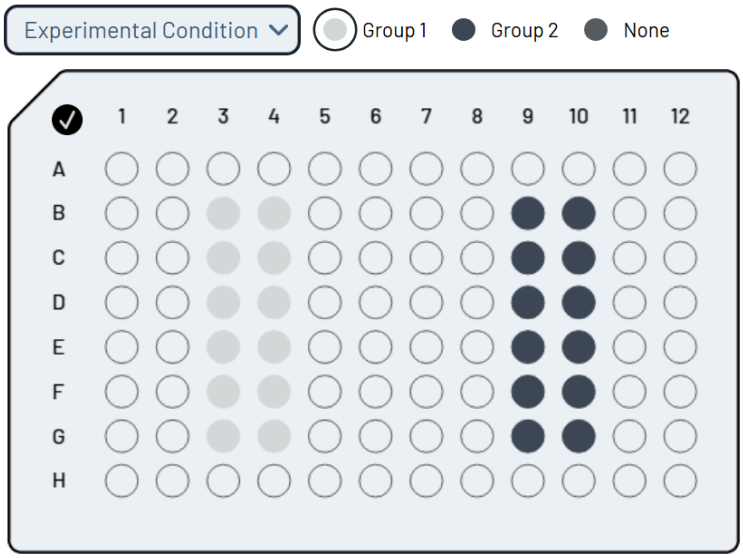
- Standard 96-well cell culture plate, cells and media
- Resipher
- ETC modulators (e.g. Oligymycin, FCCP, BAM15, Rotenone, etc.)

Methods

1. Plate cells in 96 well plate
    a. Ensure to have untreated cells with just media and wells with just media and no cells as controls
    b. Seeding density will be specific to cell type and desired OCR range
        i. Optimization of these parameters should be done prior to bioenergetic profiling

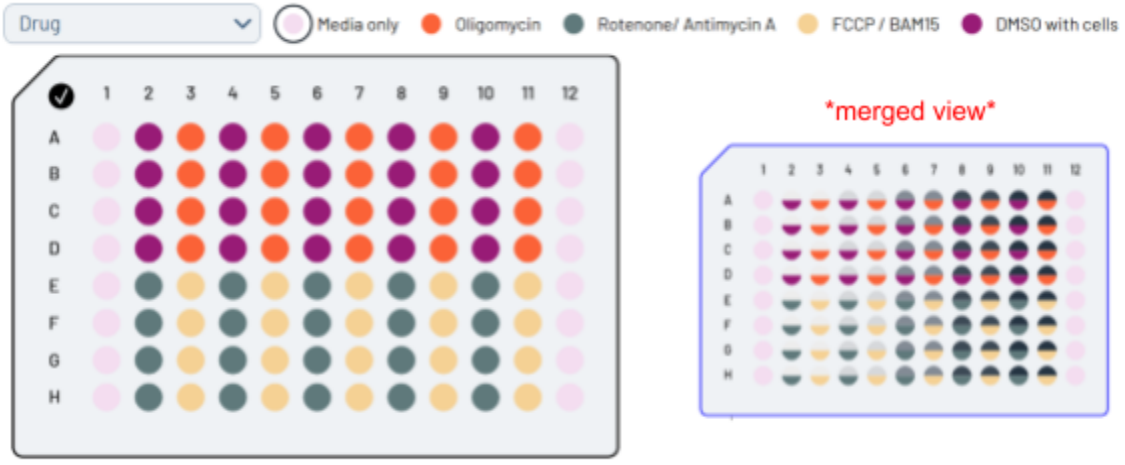
2. Suggested seeding layouts for the NS96 and NS32 Resipher are as follows:



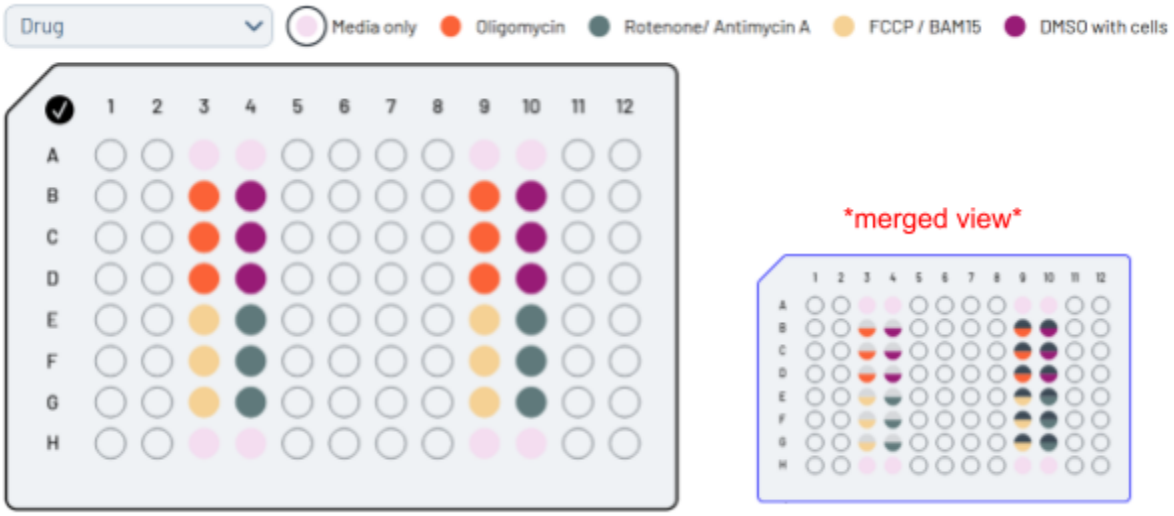


3. Allow cells to settle to the bottom/ adhere before adding Resipher sensing lid and device
4. Incubate cells with Resipher and initiate experiment to collect baseline reading
    a. Collect baseline reading overnight– this is relevant for normalization during data analysis

5. Add ETC treatments
    a. Suggested ETC treatment layouts for the NS96 and NS32 Resipher are as follows:



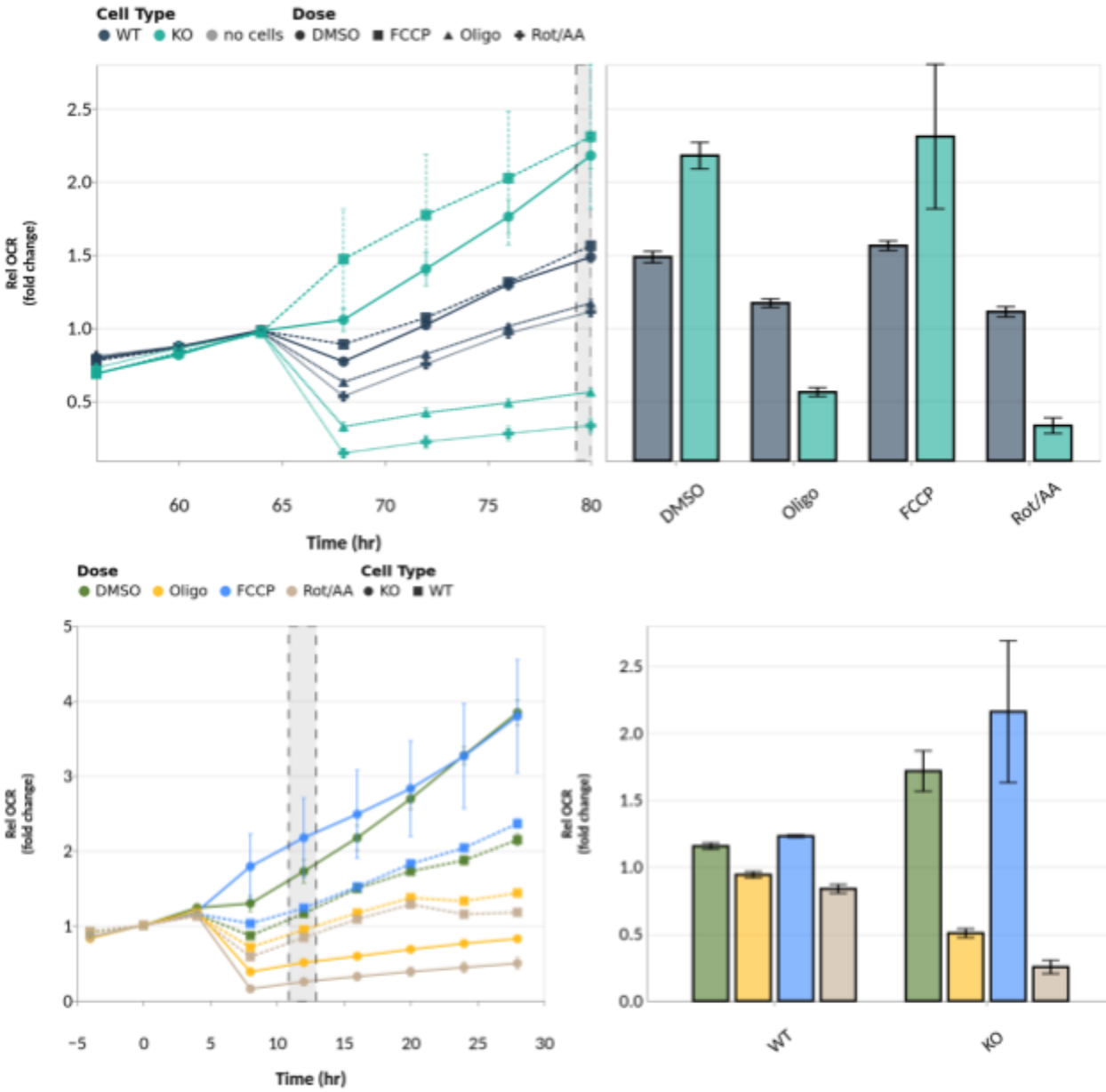
Note the NS32 layout features a reduced number of experimental groups



Optimization of ETC modulators should be done prior to bioenergetic profiling. Suggested layouts for optimization can be found in "Optimization of ETC Modulators."

Data Analysis

The results of the BEP experiment can be visualized via a Timeprint Chart under the Outputs view within the experiment.



Please visit our resources/documents section and view “Timeprint Plot Guide” for instructions.

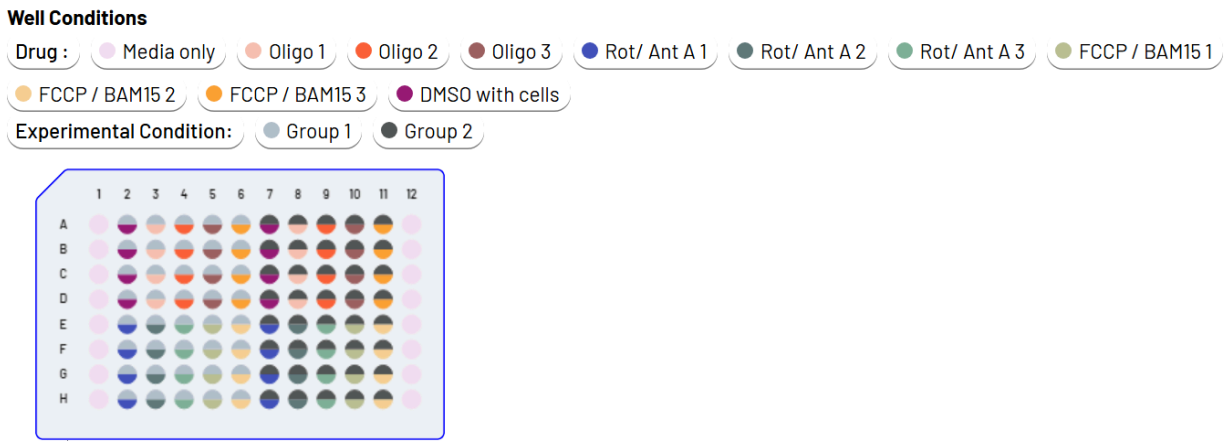
Optimization of ETC Modulators

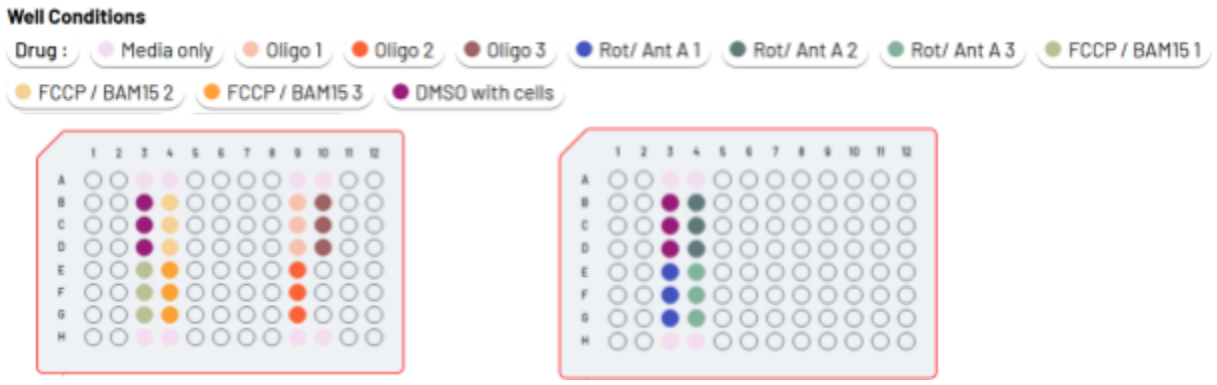
ETC modulator concentration may be dependent on cell type and seeding density. Recommended ranges for FCCP, BAM15, Oligymocyin, Rotenone are as follows:

Compound	Oligomyocin	Rotenone / Antimycin A	FCCP	BAM 15
Dosage (µM)	0.5 - 2.0	0.5 - 2.0	0.4 - 4.0	0.5 - 2.5

Testing at 3 or more concentrations of each ETC drug is recommended.

Suggested plate layout for the NS96 and NS32 are as follows:





NS32 features 2 plates to fully optimize ETC treatment dosage

Key Factors / Common Pitfalls

- Ensure all compounds and media are pre-warmed and equilibrated with incubator oxygen
    - OCR readout is sensitive to thermal and gas changes, ensuring all components are acclimatized to the experimental conditions pre-experiment ensure highest quality results
- Pre-treatment OCR level should be between 30-100 fmols/mm^2/s to allow detection of post treatment changes up or down.
- Ensure treatment media volume is not limiting maximum OCR (recommend post-treatment media volume of 70-100 µL).

References

Cloé Tessier, Maxime Toujas, Antonio C. Pagano Zottola, Andreas Bikfalvi, Thomas Mathivet, Thomas Daubon, Lucie Brisson, Audrey Burban, Ahmad Sharanek, **Protocol for real-time measurement of mitochondrial bioenergetics in 3D-cultured brain tumor stem cells using the Resipher system**, STAR Protocols, Volume 6, Issue 1,2025,1 03651, ISSN 2666-1667, https://doi.org/10.1016/j.xpro.2025.103651.

Zhang, Q., Shu, D. Y., Bryan, R. A., Han, J. Y. S., Gulette, G. A., Lo, K., Kim, L. A., Miller, J. M. L. **Long-term Monitoring of Oxygen Consumption Rates in Highly Differentiated and Polarized Retinal Pigment Epithelial Cultures**. J. Vis. Exp. (210), e67038, doi:10.3791/67038 (2024).