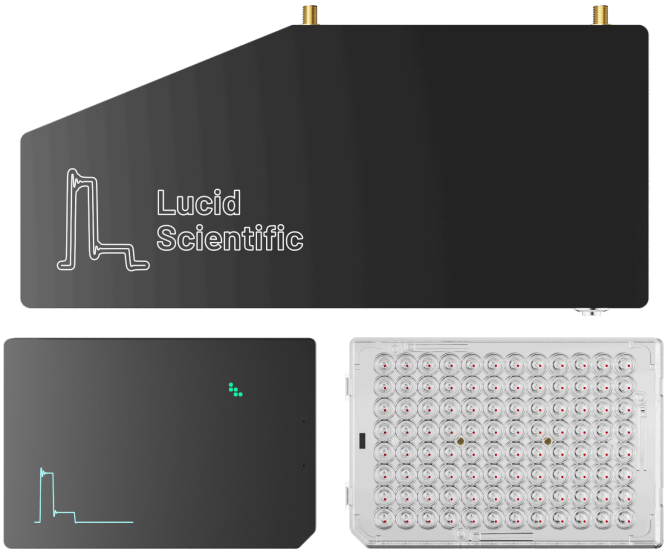
Lucid Scientific

Resipher

Quick Start Guide



This short guide provides crucial advice and tips to begin Resipher real-time continuous monitoring analysis experiments. The Resipher system will enhance your experiments in cellular metabolism by converting standard cell culture plates into smart hand-held readers that sense real-time metabolic changes. Also refer to the full user guide for detailed system and experimental setups.

This “Quick Start Guide” and the “Full User Guide” are found below:

- **Start simple**
  For your first ever experiment, we recommend starting with a simple cell density/seeding experiment. This will allow you to get familiar with the Resipher system and determine initial conditions for achieving baseline OCR readings in an optimal range for further treatments. For treatment response studies where culture densities can be adjusted, we recommend establishing a pre-treatment baseline between 30-100 fmols/mm^2/s.

- **Media and treatments should be pre-equilibrated to incubator conditions**
  The media/treatment should be pre-equilibrated to incubator conditions (e.g. 5% CO_2, 37°C prior to change) for optimal initial measurement. The oxygen environment in the media is highly dependent on temperature and gas equilibrium with the surrounding environment. For example, 100 µL unstirred media takes at least 1-2 hours to equilibrate from standard atmospheric conditions to typical incubator conditions. This equilibration time can be significantly longer for other environments and other media volumes (for example: hypoxic chambers).

- **Let Resipher (the usb handheld device) sit in the incubator for 1-2 hours prior to experiment, with sensors down on the provided Lucid plate.**

  Important:

  Do not store Resipher in the incubator outside of this warming phase or during active experiments. When not prewarming or in use, store Resipher outside of the incubator in a cool, dry environment.

- **Let the cells settle**
  It's always a good idea to allow cells to settle to the bottom of the wells before placing the sensing lids onto the well plates. This prevents cells from accidentally attaching to the sensing probes.

- **Media Volume**
  OCR can be diffusion-limited depending on the media volume. Even in standard CO_2 incubators, hypoxic conditions can be induced when the OCR demands of the cells exceeds this limit, and expected increases in cellular respiration may not be observed . For typical cultures, we recommend

Media Volume (uL)	Max OCR (fmols/mm^2/s)
65	275-325
100	180-220
150	115-140
200	90-110

starting with 100 µL. For highly aerobic cell types this volume may need to be as low as 65 µL to prevent diffusion-limited OCR.

- **Sensing Lids & Probe Lengths**
  Lucid offers varying probe lengths for different plates and different types of cell model systems (2D, 3D, organoid, tissue, etc.) For the full table with more details, please visit lucidsci.com/resources/documents:" Resipher Validated 96-Well Plates & Sensing Lid Selection Guide for Monolayer Culture." The correct plate type must be set in the plate selection dropdown in Lucid Lab during experimental set-up to ensure data is reported accurately. If the desired plate does not appear, please contact our support team at support@lucidsci.com to identify the correct lid for your experimental setups.

  Important:

  - Do not autoclave the sensing lids. Do not use hydrogen peroxide. Plates are sterile and meant for one time use.
  - The sensing material will degrade over time with multiple uses.
  - Store the sensing lids away from direct sunlight.

- **Outer wells as experimental controls**
  Edge wells may be used for controls. This reduces the likelihood of evaporation interfering with important data.

- **Evaporation is inevitable**

  PBS can be added to unused wells to reduce media evaporation during experiments. If your incubator is prone to evaporation issues, please check incubator water pan levels regularly. We have validated some 96-well plates, including the Nunc™ Edge™ 96-Well plate and Greiner Chimney well 96-well plates, that can potentially reduce evaporation.

- **Let the cells do their things**

  During an experiment, it's best to minimize disruptions, for example, opening and closing the incubator. Resipher is a sensitive instrument that responds to small

environmental changes. Please reach out to us if you have not received a magnet with your **Resipher** to let others know that you are running an experiment and noting not to open the door.

Key customer publications & protocols:

Tan J, Virtue S, Norris DM, Conway OJ, Yang M, Bidault G, Gribben C, Lugtu F, Kamzolas I, Krycer JR, Mills RJ, Liang L, Pereira C, Dale M, Shun-Shion AS, Baird HJ, Horscroft JA, Sowton AP, Ma M, Carobbio S, Petsalaki E, Murray AJ, Gershlick DC, Nathan JA, Hudson JE, Vallier L, Fisher-Wellman KH, Frezza C, Vidal-Puig A, Fazakerley DJ. ***Limited oxygen in standard cell culture alters metabolism and function of differentiated cells.*** *EMBO J. 2024 Jun;43(11):2127-2165. doi: 10.1038/s44318-024-00084-7. Epub 2024 Apr 5. PMID: 38580776; PMCID: PMC11148168.*

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.*

Hass DT, Zhang Q, Autterson GA, Bryan RA, Hurley JB, Miller JML. ***Medium Depth Influences O2 Availability and Metabolism in Human RPE Cultures.*** *Invest Ophthalmol Vis Sci. 2023 Nov 1;64(14):4. doi: 10.1167/iovs.64.14.4. PMID: 37922158; PMCID: PMC10629522.*

Triolo M, Khacho M. ***Protocol to monitor live-cell, real-time, mitochondrial respiration in mouse muscle cells using the Resipher platform****. STAR Protoc. 2024 Dec 20;5(4):103330. doi: 10.1016/j.xpro.2024.103330. Epub 2024 Sep 20. PMID: 39305486; PMCID: PMC11459069.*