Reducing Equilibration Time

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Overview

Resipher is commonly utilized for monitoring the oxygen consumption rate (OCR) of cells over extended periods, ranging from a day to weeks. To maintain optimal cell conditions and ensure their well-being, it is crucial to add culture media regularly. Additionally, researchers often seek to assess treatment responses during regular monitoring periods.

In both scenarios, the Resipher must be temporarily removed from the sensing lid and well plate to allow for media change and/or treatment addition. This temporary removal is necessary because Resipher measures OCR uniquely by analyzing the media oxygen concentration gradients within each well. To obtain accurate consumption analyses, these gradients need to form, requiring a brief equilibration period after every plate movement or addition of substances.

Minimizing disruptions to the Resipher probes is paramount to obtaining reliable data. To help shorten the possible equilibration time, we have compiled and outlined some best practices in this document. It's important to note that the effectiveness of these practices may vary depending on factors such as cell types, media compositions, and specific treatments used in a specific experiment.

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Media and/or Treatment Additions

Equilibrating the media

- Place the entire container of media in the incubator with the cap slightly loosened overnight; this will allow the media dissolved gas content (i.e. 5% CO_2) and temperature to equilibrate.

- For a series of treatments in different wells, you may prepare them in a new 96-well plate; add treatments into specific wells and place the covered well plate into the incubator overnight to equilibrate. This allows for ease of treatment addition using a multi-channel micropipette.

Addition of Media or Treatment

- Carefully lift and remove the Resipher device away from the sensing lid, with the Resipher sensing lid still sitting on the 96 well plate, transfer the plate to the biosafety cabinet for media change and treatment addition. Make sure a lid is always covering the well plate when it is not inside a sterile environment such as a biosafety cabinet. The same applies to the container of media and the prepared treatments in the other 96-well plate; always handle media and cells in a sterile environment such as a biosafety cabinet.

- In the biosafety cabinet, liftthe Resipher sensing lid from the well plate. During media or treatment changes, the sensing lid can sit in the biosafety cabinet with the sensing probe tips pointing up; the sensing lid can also be placed onto another empty multiwell plate during media change.

- After the media or treatment change is finished, place the Resipher sensing lid back onto the well plate and return it to the incubator. Attach the Resipher device back onto the sensing lid.

- Make sure the status light on the Resipher device shows a solid green light; check O2C data collection resumes on the Lucid web app.

Summary

The guide should aid Resipher users in shortening equilibration time to a few hours. If you are seeing the desired effects, please reach out to our support team (lab@lucidsci.com) for further discussion on experiment setups.

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Other Useful Resipher Tips

- **Start simple:** For your first Resipher experiment, we recommend you start with a simple experiment, for example, a cell density/seeding experiment. It allows you to get familiar with the Resipher system and confirms the general oxygen consumption behavior of the cells before proceeding to more complex experiments. If the data is looking good during the first few days of the experiment, then consider applying treatments.

- **Let the cells settle:** It's always a good idea to allow cells to adhere or fall 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.

- **Temperature is important**: Sensors are cross-sensitive to temperature. Media should be pre-equilibrated to 37C before using to reduce initial stabilization period. Temperature gradients may appear as a negative OCR.

- **100 uL media recommended to start**: Commonly used media volumes range from 75 to 200 uL. Too little media (below 60 uL) may risk the probe moving out of the media. Due to the diffusion limit of oxygen in media, higher volumes means lower theoretical maximum oxygen consumption rates (For example, 180 fmmol/mm2/s for 100 uL and 100 fmmol/mm2/s for 200 uL). Plus, the cells may experience a more hypoxic environment with greater media depths. Unless other volumes are required, we typically recommend starting with 100 uL and make volume adjustments in future experiments.

- **Don't "lose" control:** We recommend at least one, possibly more media only wells.

- **Limit evaporation:** Media or PBS can be added to unused wells to reduce media evaporation during experiments. Watch out for edge effects where evaporation rate is usually faster.

- **Data Review:** We encourage researchers to contact us for data review meetings - especially after initial experiments. Please reach out to: lab@lucidsci.com for any support and help from our team.