

Reducing the Impact of Media Evaporation

APRIL 2024



Resipher is commonly utilized for monitoring the oxygen consumption rate (OCR) of cells over extended periods of time, ranging from days to weeks in many instances. With Resipher, users can use most 96 well plates from various manufacturers.

Resipher is uniquely designed to be an “open-air” setup, where oxygen is continuously supplied to the media and cells, thus allowing for long-term OCR monitoring. A challenge one may encounter during long-term cell cultures is media evaporation. Evaporation usually impacts edge wells to a greater extent due to their closer proximity to the ambient incubator environment. Limiting the evaporation impact will enable the accuracy and reliability of Resipher data. Here are a few strategies one can employ to limit the impact of media evaporation.

Fill unused wells with PBS

When using a 32-channel Resipher system, the working wells are in columns 3, 4, 9, and 10. If the rest of the wells are not used for other analyses, it is always a good idea to fill them with PBS to help keep the air volume under the sensing lid as humid as possible.

Use well plates with surrounding moats

There are well plates that are engineered to reduce media evaporation. For example, Thermo Scientific™ Nunc™ Edge 2.0 Plate (Cat# 167574) has surrounding moats along the outer edges of the well plate. When filled with PBS, these moats can act as evaporation buffers during extended Resipher experiments.

Ensure the water pan is not dried out:

It is always a good idea to check the water pan inside the incubator periodically to make sure it is not totally dried out.

Limit access to incubator during Resipher experiments

Keeping the incubator door closed as much as possible during Resipher experiments will limit humidity fluctuation. Another important added benefit - it will also ensure a constant temperature and OCR data stability.

Reducing the Impact of Media Evaporation

APRIL 2024



General 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 37 C 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.