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Who can use the UNC NORC Seahorse services?

Anyone can use our service.

Can users outside UNC access the Seahorse services?

Yes, external users can register in iLab to use the Seahorse services.

Can I operate the Seahorse Analyzer myself?

No; at this time, we offer Seahorse as a service in which UNC NORC staff will run your assay for you. This protects our XFe96 from damage and gives you the best quality data.

How does extracellular flux analysis work?

At the UNC NORC, we use an XFe96 Extracellular Flux Analyzer from Agilent Seahorse. The XFe96 measures the oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) in a 96-well plate. OCR is an indicator of mitochondrial respiration, and ECAR is largely the result of glycolysis. For more information, please visit Agilent's website.

How do I book my Seahorse experiment?

You will submit your service request through iLab. Core staff will respond to confirm your scheduling and details.

What is the cancellation policy?

If you need to cancel your booking, as long as you let us know 24 hours before your scheduled appointment, you will not be charged. If you cancel with less than 24 hours' notice, you will be charged for any supplies or time incurred preparing for your assay.

What is the fee structure for Seahorse XFe96 experiments at the UNC NORC?
It depends on the assay you are going to use and the supplies you need. Standard assays range from about $400 to $500. Please see our Services list in iLab for detailed prices.

Can I use a portion of the cartridge plate or reuse it?
Unfortunately, the XFe96 is designed to accept a cartridge plate only once, so it's all or nothing. You can use only a portion of the 96 wells, but the cost of the experiment will be the same, and you can't use the remaining wells at a later date. Even if the instrument could accept the cartridge twice, the sensors are only good for about 72 hours post hydration.

How do I possibly fill an entire 96-well plate?
We recommend having 3-6 technical replicates of each condition represented on the same plate; this will improve your data quality and give you better statistical data.

What is required from the user?
The Core staff will do everything for you; you will only need to select your assay, plan your plate layout, and prepare the cells by seeding, washing, and bringing them to us.

How do I know at which density to seed my cells?
The Agilent website has a fantastic tool – the Agilent Life Science Publication Database – that provides an easy way to search scientific publications that reference Seahorse XF data. Simply enter your cell type or cell line, click submit, export the results to Excel, and look for papers that have completed similar Seahorse experiments on 96-well plates. This will give you an idea of what density to start at. However, because everyone's techniques and cells are different, we recommend that your first experiment includes optimization for cell density. To do this, we recommend testing 3-4 densities with a minimum of 3-6 technical repeats of each.

How do I know what concentration of Oligomycin or FCCP to use in my assay?
Agilent used to recommend optimizing for oligomycin concentration in the same way we optimize for cell density. From our experience (and that of Agilent), 1 uM oligomycin is usually ideal, but this optimization can easily be combined with cell density optimization to improve the quality of your data.

However, every cell type seems to require a different concentration of FCCP to achieve the greatest signal. Since you want to know the true maximal respiration of your cells, you want to be working at the optimal FCCP concentration. Too low, and the signal will be too low; too high, and the signal will still be too low. Thus, we recommend performing a six-point FCCP titration (0, 0.125, 0.25, 0.5, 1.0, 2.0) with your optimal cell density as part of your first mito stress test experiment. This is the protocol Agilent recommends.

If you truly want to compare max respiration for each different cell line (e.g., WT vs. mutant), you may want to titrate FCCP for all cell lines. We realize this can become onerous and expensive – we will do our best to create the most efficient and cost-effective experiments for you. The good news is that most people get usable data even from these initial optimization experiments.

How do I acquire Wave software?
Agilent Seahorse Wave Desktop software is available for download from the Agilent Seahorse website.

How can I receive my Seahorse results?
The Core staff will set up a folder for your lab on OneDrive, where your results will be uploaded and shared with you for downloading.

How do I acknowledge the UNC NORC in my presentations or publications?
Please acknowledge NIH grant P30DK056350 in all publications resulting from the use of NORC services. https://norc.unc.edu/about-us/cite-the-unc-norc/.

Can I meet with someone to discuss my Seahorse experiment?
Yes, we would be happy to meet with you and discuss your Seahorse experiment. However, know that we are not experts in your cell type or metabolic question. You may want to reference the Agilent Life Science Publication Database for information specific to your cell type and/or selected assay.

We recommend the Seahorse Technical Support Line for technical assay questions: 800-227-9770, option 3, option 8.