Technical Overview

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Explore Metabolism Where You Couldn't Before

Delivering higher sensitivity using fewer cells with the Agilent XF HS miniplate

Abstract

Agilent Seahorse XF users are increasingly investigating more challenging and complex, biological models such as rare, primary, and isolated cell populations. However, there are often limitations in the quantities of these cells that can be acquired, which results in fewer sample replicates or poor data quality due to low cellular signals. The Agilent Seahorse XF HS miniplate allows users to obtain the same information about the two main cellular bioenergetic pathways (mitochondrial respiration and glycolysis) as traditional Agilent Seahorse XF assays but uses three times fewer cells per well. Dedicating less sample to each well increases the number of replicates that can be incorporated into each experiment, providing the statistical relevance needed to more confidently interpret data from each cellular preparation while maximixing the amount of high quality XF data generated.

The enhanced data quality provided by the Agilent XF HS Mini Analyzer together with the higher sensitivity of the XF HS miniplate and the workflow improvements produced by precoated Poly-D-Lysine miniplates deliver a simple, high performance, purpose-built suspension cell workflow ideal for when biological material is limited. This workflow delivers the ability to discriminate low bioenergetic rates where it was previously not possible.

Introduction

Agilent Seahorse XF technology measures cellular oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) at higher sensitivity than any other technology. This sensitivity is possible due to the creation of a transient microchamber during instrument measurements. When a measurement is performed, the instrument lowers the cartridge probe, with it's embedded oxygen and pH sensors, into the assay well. The sensors are positioned 200 µm above the well bottom, forming a transient closed microchamber of a few microliters. As the oxygen and pH levels change, the extracellular flux of these analytes is detected by the sensors and read by the instrument. Measurements are typically made for 3 minutes and rates are calculated in real time. Upon completion of this measurement period, the probes are raised allowing the extracellular medium to revert to baseline conditions. Due to the small volume of the microchamber, the changes in oxygen level and extracellular pH produced by the cells attached to the bottom of the well are "magnified" compared to the changes produced if the measurements were performed in the total well volume enabling convenient, robust metabolic interrogation of your cell type of interest.

Agilent Seahorse XF HS miniplates feature a molded ring structure on the bottom of the miniplate (Figure 1). This structure, together with a silicone mask insert, focuses cells into a smaller area and, on formation of the microchamber, reduces the measurement volume to 1/3 of the standard XFp cell culture miniplate (Figure 2). This reduction results in a three-fold increase in measured signal when using the same quantity of cells, in turn facilitating the measurement of three times fewer cells.

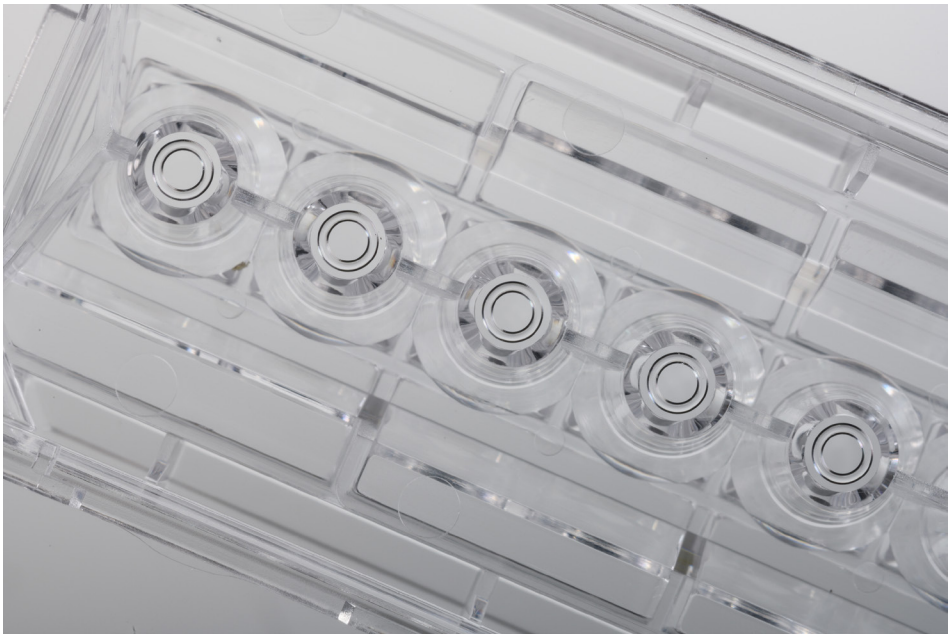


Figure 1. The Agilent XF HS miniplate incorporates a newly designed "ring" feature. This ring confines the cells to a smaller surface area, thereby forming a smaller microchamber volume. The reduced surface area results in a higher signal-to-noise ratio where the magnitude of the "signal" detected is higher when using the XF HS miniplate. By providing higher sensitivity when detecting the signal, accurate and confident XF measurements are possible using fewer cells per well.

These improvements in sensitivity are illustrated in Figure 3, which shows a comparison of the oxygen and pH signals achieved using the same number of cells in each miniplate format. At the beginning of each measurement cycle, probes lower to form a transient microchamber at the bottom of each cell well, during which time the instrument measures sample pH and oxygen partial pressure. Metabolic activity causes oxygen depletion and media acidification within the microchamber, both of which are measured in real-time by the Seahorse XF system, and subsequently used to calculate Proton Efflux Rate (PER) and Oxygen Consumption Rate (OCR), taking microchamber volume and other related factors into account. This calculated rate is represented by a single data point on the kinetic OCR/PER trace. As described above, in comparison to XFp miniplates, XF HS miniplates focus cells into a smaller area and generate a much smaller microchamber volume, thereby delivering more pronounced acidification and oxygen depletion from a given number of cells. This is observed as steeper oxygen depletion and acidification profiles. The system accounts for the reduced sample volume, so, correctly calculated OCR and PER rates do not change (as indicated by the red box in Figure 3), however as the XF HS miniplate generates a higher magnitude of oxygen depletion and acidification, OCR and PER can be calculated with greater confidence such that the lower levels of metabolic activity become measureable, fewer cells can be used, and more subtle rate differences can be observed. Table 1 shows the recommended basal metabolic rate ranges using either the XF HS miniplate or XFp miniplates with the XF HS Mini analyzer.

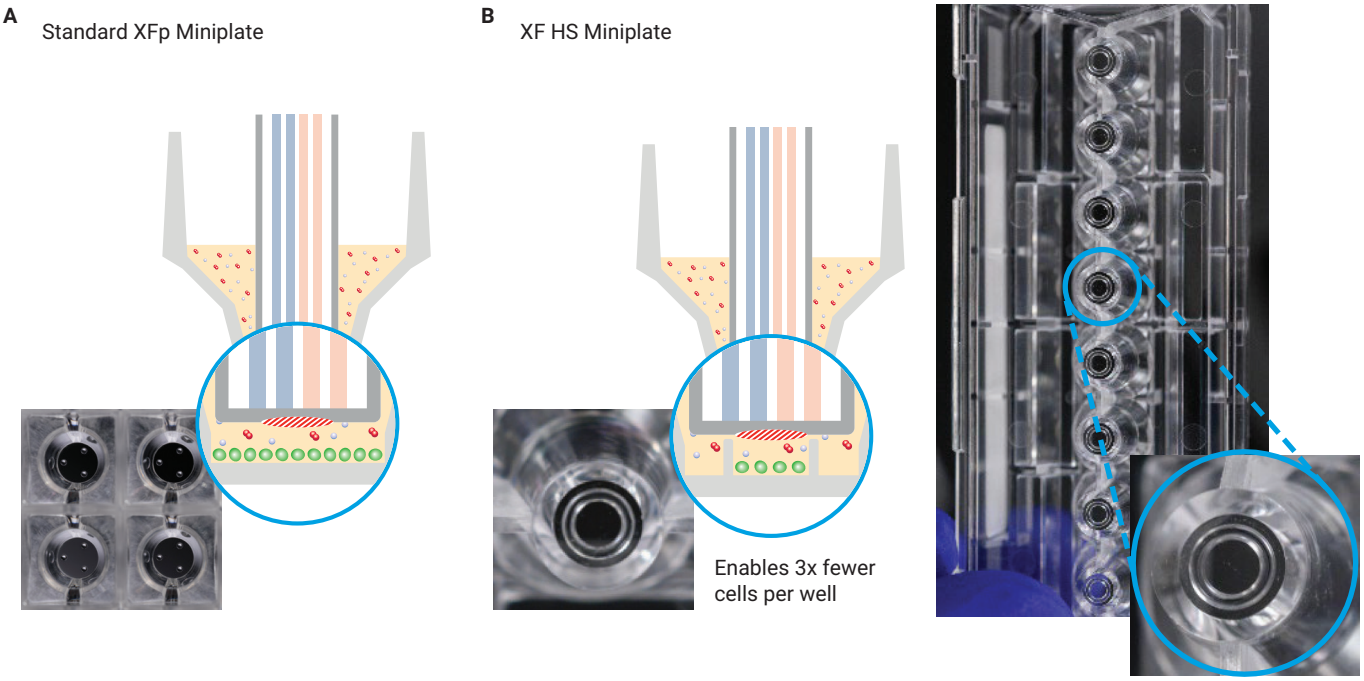


Figure 2. A zoomed in image of each microchamber type is displayed. (A) The standard Agilent Seahorse XFp miniplate microchamber (2.28 µL). (B) The Agilent Seahorse XF HS miniplate microchamber (0.76 µL).

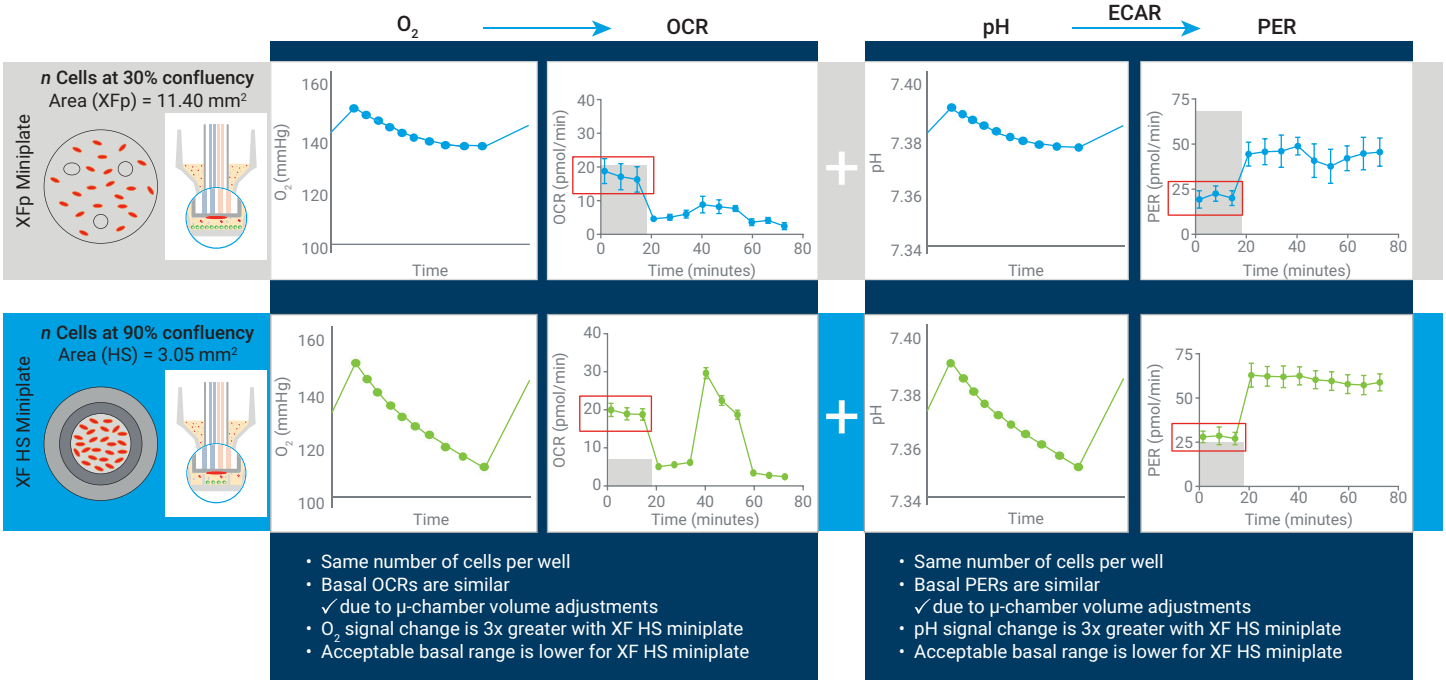


Figure 3. Agilent Seahorse XF HS miniplate signal amplification. The Agilent Seahorse XF Mito Stress Test Kit was run on the Seahorse XF HS Mini Analyzer with THP-1 cells seeded at 12,000 cells/well on both Agilent XFp miniplates and XF HS miniplates. The data sets show a comparison of the signal levels (oxygen consumption and pH) detected using either plate type and the subsequent transformation of signal level data to metabolic rates. Areas in gray indicate basal ranges that are not in the recommended metabolic rate range. The O_2 consumption and H^+ excretion levels are increased three-fold in magnitude using the XF HS miniplate.

Note: The lower XF HS miniplate microchamber volume delivered higher Extracellular Acidification Rate (ECAR) values from a given number of cells, in comparison to the XFp miniplate. This is because ECAR values describe changes in pH per unit time, and therefore do not account for measurement volume, such that when microchamber volume is reduced, ECAR increases proportionally. In contrast Proton Efflux Rates (PER) values are provided in concentration units (pmol H^+/min) and so account for sample volume in a similar manner to OCR measurements.

Seahorse XF recommended basal rate ranges

Agilent Seahorse XF guidance allows researchers to diagnose the quality of their experiments through interpretation of the basal metabolic rate range. Independent of cell type used, researchers are now able to correlate data quality with the optimal seeding density.

Table 1. Seahorse XF recommended basal rate ranges (Agilent Seahorse XF HS Mini Analyzer with two different plate types).

Target Basal Metabolic Rate Ranges		
	Miniplate Type	
	XF HS	XFp
OCR (pmol/min)	7 to 55	20 to 165
ECAR (mpH/min)	10 to 90	10 to 90
PER* (pmol/min)	25 to 228	68 to 610

* PER range was calculated with a buffer factor of 2.5. PER and the range will vary based on the buffer factor of the cartridge used during the XF analysis.

Materials

FluxPak contents

Agilent Seahorse XF HS Mini FluxPak
(part number 103723-100)

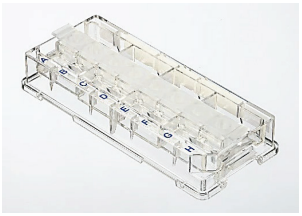
1. XF HS miniplates (12 plates)
2. XFp sensor cartridges (12 sensor cartridges) with utility plates
3. Bottle of calibrant (100 mL)

Agilent Seahorse XF HS Mini FluxPak-PDL miniplates
(part number 103724-100)

1. XF HS-PDL miniplates (12 plates)
2. XFp sensor cartridges (12 sensor cartridges) with utility plates
3. Bottle of calibrant (100 mL)

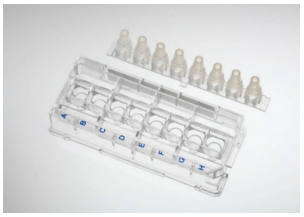
XF HS miniplate handling

Pre-assembled XF HS miniplate with silicone mask



Removal tool

XF HS miniplate with silicone mask removed



Sample preparation

XF HS miniplate sample preparation adheres to the same steps as other Agilent Seahorse assays with a few extra considerations. The infographic in Figure 4 shows an illustrated overview of the sample preparation process.

Preparing an XF HS PDL miniplate for an XF assay

The day before the XF assay

1. Hydrate the eight-well sensor cartridge and place it in a 37 °C non-CO_2 incubator, overnight.
2. Warm the XF HS PDL miniplate in a non-CO_2 incubator, overnight.

On the day of the XF assay

3. Perform calibrant exchange with the eight-well sensor cartridge and keep in an incubator until ready for XF assay.
4. Warm XF medium in a 37 °C water bath and supplement as required.
5. Seed XF HS PDL miniplate with desired cell quantity per well in XF medium.
6. Spin plates in a centrifuge to immobilize cells onto PDL.
7. Remove XF HS miniplate cell seeding mask using the mask removal tool.
8. Add 160 µL XF medium to bring the final volume in the plate to 180 µL.
9. Observe the cells under a microscope to ensure confluency and immobilization. Keep the plates warm until ready for XF assay.
10. Prepare XF assay kit reagents and load eight-well sensor cartridge as required.
11. Begin cartridge calibration on XF HS Mini Analyzer.
12. When calibration is complete, load the XF HS miniplate seeded with cells. Ensure that the cell seeding mask and plate lid are removed before loading the plate in the analyzer.

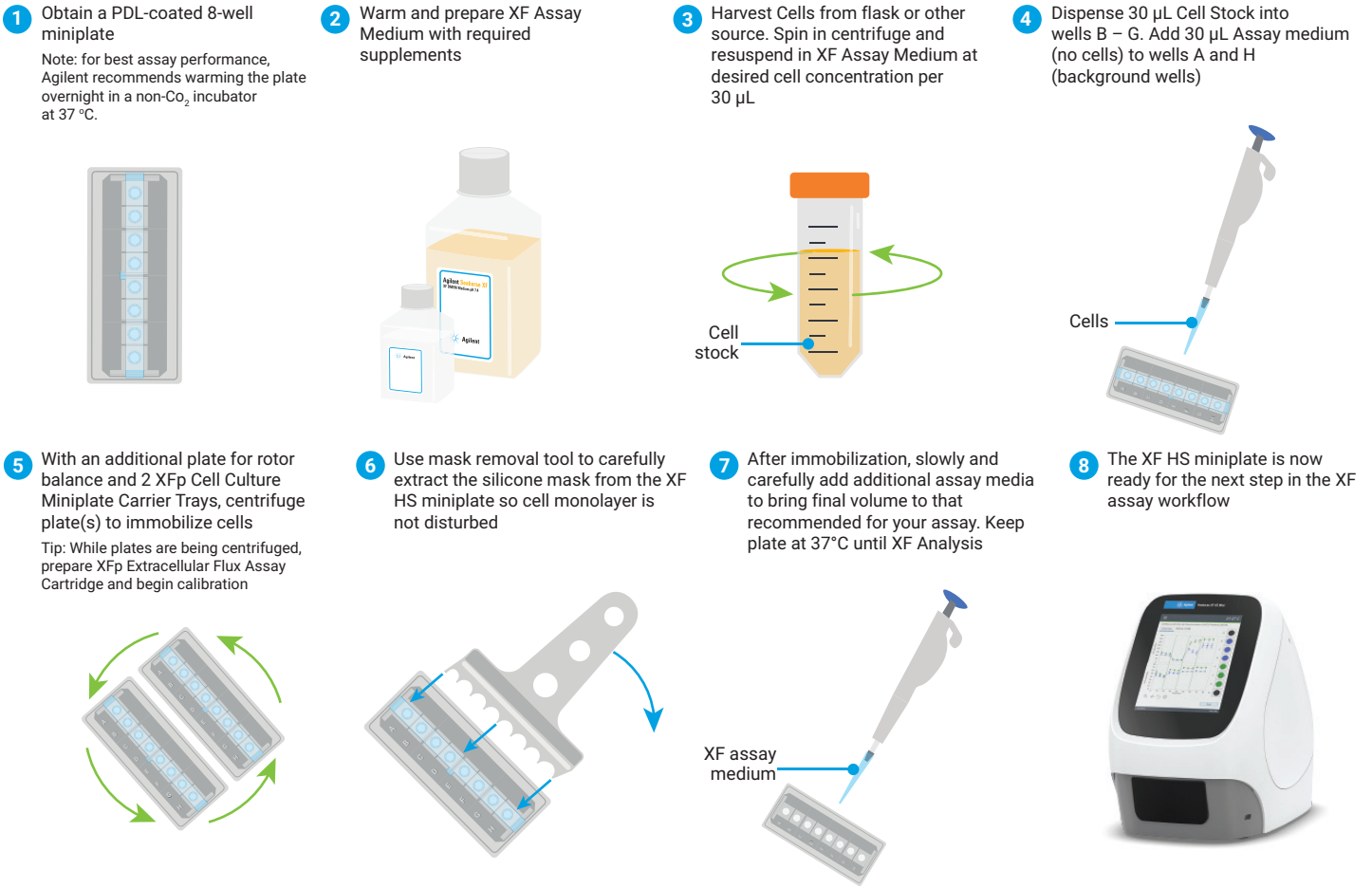


Figure 4. An illustrated overview of sample preparation for Agilent Seahorse XF assays using the Agilent Seahorse XF HS miniplate.

Important tips and tricks

Due to the change in microchamber size and the addition of the cell seeding mask, there are a few important considerations when using XF HS miniplates.

1. **Cell seeding:** The XF HS miniplate cell seeding surface is about 33% of the area of an XFp or XF96 microplate well. When comparing to XFp or XF96 microplates, use about 1/3 the number of cells in the XF HS miniplates. Optimal cell numbers will vary by cell type. In general, adherent cells are best seeded between 1.0 and 10.0 × 10^3 cells/well, while suspension cells are generally seeded between 2.0 and 7.0 × 10^4 cells/well.
2. **Pipette handling:** The cell seeding mask creates a smaller well in the eight-well cell culture plate. This introduces some challenges in cell seeding. Unlike standard XFp miniplates, when seeding the XF HS miniplates, the pipette tip should be inserted to just about the bottom of the well. If the pipette tip is only partially inserted, the cells and media dispensed will be stuck in the well with a bubble of air below. However, this is generally mitigated once the plates are centrifuged. After centrifuging, be sure to check each well to ensure that cells have been immobilized on the surface of the well.
3. **Removal of the mask:** The cell seeding mask allows cells to be seeded only in the center portion of the well. The mask must be removed to accommodate the sensor cartridge before the XF assay. One reusable mask removal tool is provided with each box of XF HS miniplates. The cell seeding mask should be removed only when cells are sufficiently immobilized, and the user is ready to prepare the plate for assay. For best results, the mask should be removed in a single effort. The prongs should be inserted between each well, beneath the mask. Ensure that the prongs are parallel to the top surface of the plate while inserting the tool and lever the tool when ready to remove the mask, holding the plate steady with one hand. Once the mask begins to lift from the plate, hold the mask onto the tool so that it does not drop back into the plate. There will be about 20 µL of media left in each well. Finally once removed, dispose of the mask.

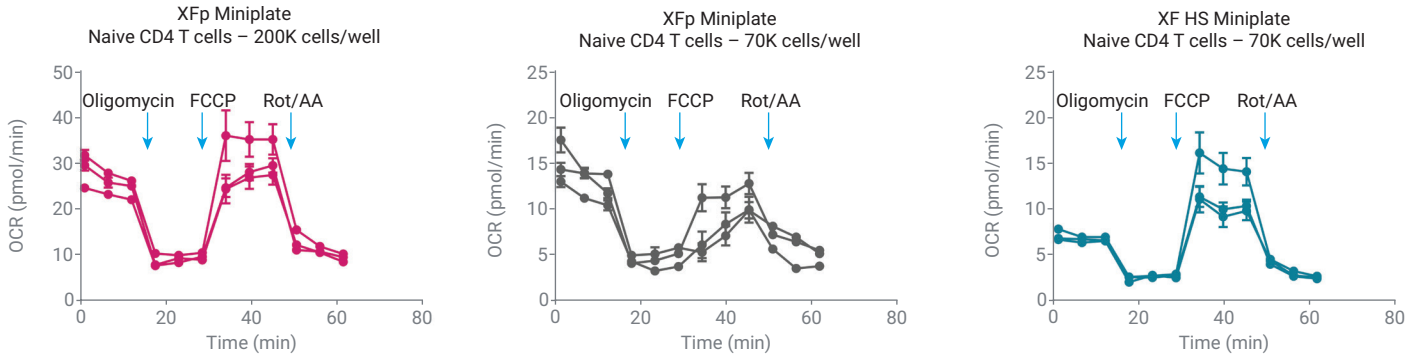
For more detailed instructions, see the following protocols:

"Seeding Adherent Cells in Agilent Seahorse XF HS Miniplates" Access Protocol Here

"Seeding Suspension Cells in Agilent Seahorse XF HS PDL Miniplates" Access Protocol Here

Analysis

A **Raw OCR data**



B **Normalized data**

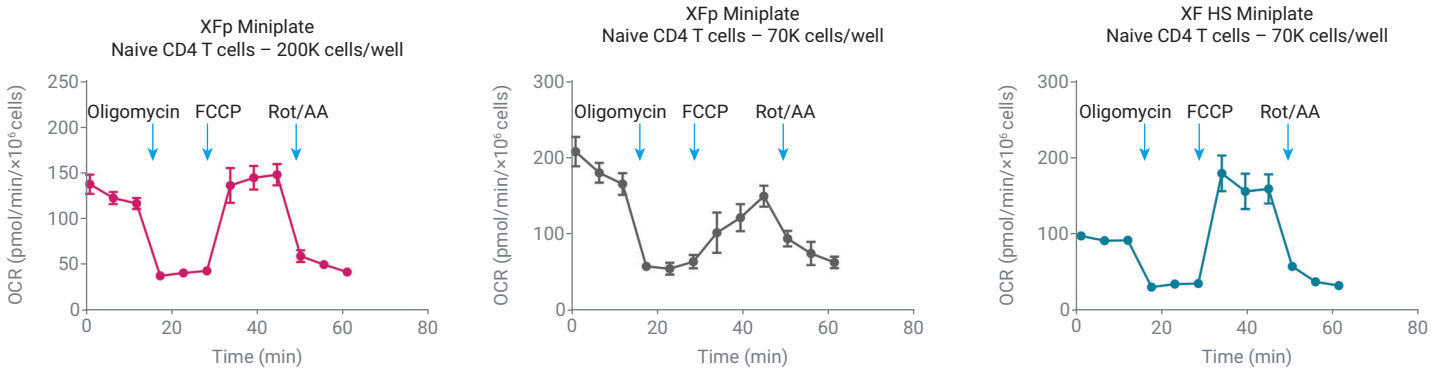


Figure 5. Agilent Seahorse XF Cell Mito Stress Test of Naïve CD4+ T cells (200,000 cells/well or 70,000 cells/well as indicated) seeded in XFp PDL miniplates (part number 103722-100) or XF HS PDL miniplates (103727-100) using Agilent Seahorse XF RPMI medium, pH 7.4 supplemented with 10 mM glucose, 2 mM glutamine, and 1 mM pyruvate. FCCP concentration used for all the assays was 1.5 µM. Normalized data represents the average of three plates ± SEM.

Conclusion

Academic researchers working with primary immune cells, sorted cell types, other primary cell types, or rare ex vivo cell populations are often limited to a finite amount of sample when performing an XF analysis. The isolation and purification procedures for these cell types are costly and labor-intensive, which prevents many researchers from accessing this routinely used, standard analysis technique for measuring cell metabolism. Furthermore, many cell types inherently exhibit low metabolic rates, which makes acquiring measurements challenging.

The XF HS miniplate is a validated cell culturing platform designed for performing metabolic measurements with fewer cells or on cells with low metabolic rates. It also lessens the pre-XF assay burden by reducing the number of animals involved or the amount of cell vials used before isolating or purifying the cells. Together the Agilent Seahorse XF HS Mini analyzer and Agilent Seahorse XF HS miniplate enable the collection of highly sensitive, accurate metabolic rate data in challenging and complex biological models that were previously not amenable to these types of valuable metabolic analyses.

www.agilent.com/chem/hsmini

RA45344.6407291667

Printed in the USA, March 8, 2024
5994-3033EN

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