Technical Overview

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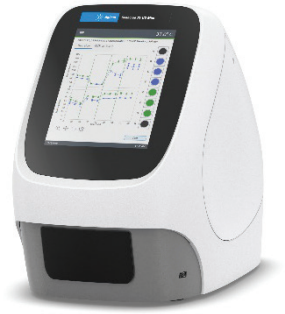
Robust T Cell Activation with Improved Data Quality and Workflow using the Agilent Seahorse XF HS Mini Analyzer



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Measure immune cells more confidently and access your data from anywhere

The Agilent Seahorse XF HS Mini Analyzer enables researchers to profile cellular metabolism with greater flexibility and reliability than ever before, particularly for nonadherent cells or cells with low levels of aerobic metabolism. Researchers can then access these new insights from anywhere, as data generated by this eight-well XF Analyzer are analyzed and interpreted using Seahorse Analytics—a powerful new web-based data analysis application that enables remote, intuitive, Mac- and PC-compatible XF data analysis for all XF Analyzer users. The Seahorse XF HS Mini solution is therefore ideally suited to research areas such as immunology, where convenient, consistent cell immobilization is required, and where many cell types exhibit a low dependency on aerobic respiration due to quiescence, inherent metabolic poise, or metabolic dysfunction.

Improved suspension cell workflow and robust T cell activation measurements

Consistent cell seeding is critical to the success of any cell-based assay, and this is particularly true when interrogating cell metabolism. For XF-based analysis of adherent cells, parameters such as seeding density are optimized to ensure maximal assay performance, while detailed metabolic interrogation of nonadherent cells is enabled by consistent, efficient cell immobilization. This is typically achieved by precoating XFp miniplates with substrates such as Poly-D-Lysine (PDL). However, day-to-day and lab-to-lab variability in coating efficiency complicates assay optimization and can negatively impact measurement repeatability. The XF HS Mini workflow addresses this through the provision of new XFp PDL miniplates (p/n 103722-100) and corresponding XFp FluxPaks (PDL miniplates) (p/n 103721-100). These miniplates deliver a significant workflow enhancement for suspension cell measurements by eliminating the PDL-coating step, reducing assay setup time, simplifying assay optimization, and delivering greater interassay consistency for both OCR and ECAR/PER measurements. These improvements are particularly relevant in immune cell applications, where limited biomaterial can often restrict the scope for extensive optimization or repeat measurements. In short, these improvements allow researchers to focus on data outputs rather than assay optimization.

This improved XF HS Mini workflow is compatible with all Seahorse XF assay kits, thereby delivering more robust, more repeatable interrogation of suspension cell metabolism. Of particular relevance is the new XF Hu T Cell Activation Assay Kit (p/n 103759-100), which enables real-time temporal analysis of T cell activation by monitoring changes in glycolytic activity. Figure 1 illustrates the profound increase in glycolytic activity that occurs upon activation and illustrates how the new Seahorse XF HS Mini Analyzer can be used to conveniently investigate modulators of T cell activation.

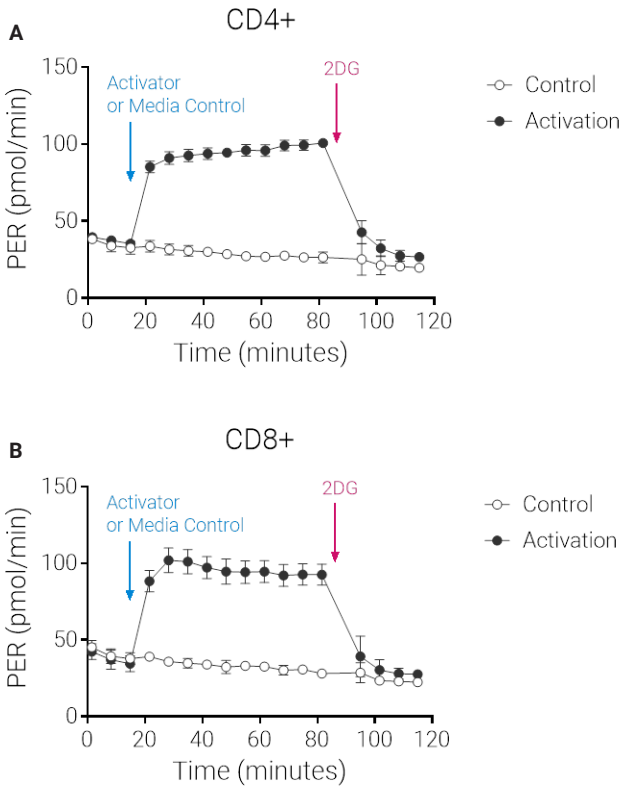

Figure 1. Naïve CD4+ (A) and CD8+ (B) T cells (200,000/well), were treated with assay media (Vehicle) or soluble CD3/CD28 activator using an Agilent Seahorse XF Hu T Cell Activation Assay Kit Pack (p/n 103759-100). The assay was run using Seahorse XF RPMI medium, pH 7.4 (p/n 103576-100) supplemented with 10 mM glucose, 2 mM glutamine, and 1 mM pyruvate.

Tight standard deviations at low OCR rates

In addition to the workflow improvements delivered by the XFp PDL miniplates, the XF HS Mini Analyzer also delivers improved oxygen consumption measurement precision at low OCR (≤40 pmol/min), resulting in a 40% reduction in measurement variability and reduced outlier rates (Figure 2).

More robust XF data quality at low OCR measurements

Together with XFp PDL miniplates, this increased performance delivers improved interassay consistency, particularly at low basal OCR (≤40 pmol/min). This interassay consistency and increased profile resolution can be seen in Figure 3, which shows the results of three independent XF Cell Mito Stress Test assays run on naïve CD4+T cells using XFp PDL miniplates and the XF HS Mini Analyzer.

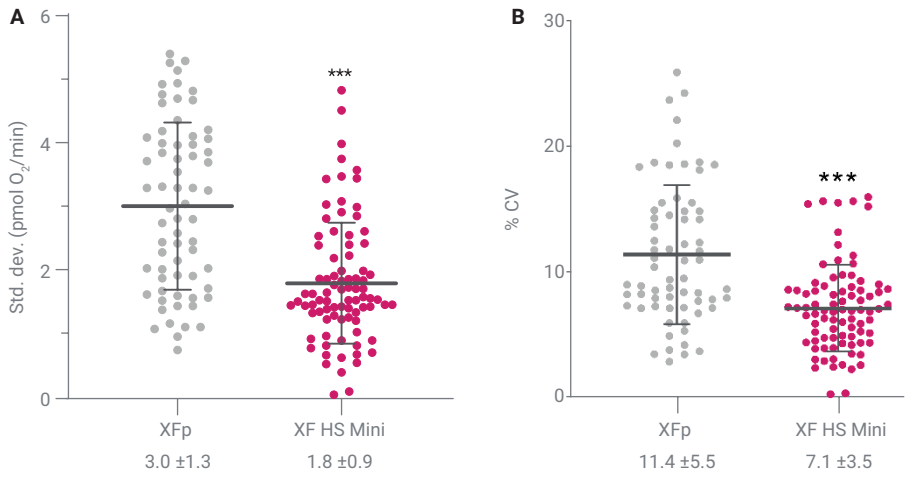

Figure 2. Standard deviation (std. dev.) and % coefficient variation (% CV) per group (3 to 6 replicate wells per group) of oxygen consumption rate (OCR) measurements below 40 pmol/min. Data were obtained from 16 independent Seahorse XF assays performed in both XFp and XF HS Mini Analyzers. SD and %CV for each time point of the assay for all the assays were combined and filtered to only include data points with OCR under 40 pmol/min. The graphs represent the dispersion of std. dev. (A) and % CV (B) obtained. *** p <0.0001 (paired t-test).

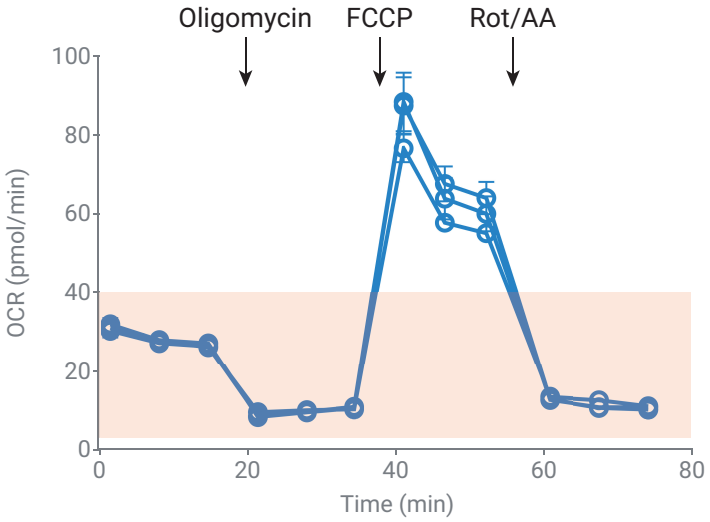

Figure 3. Agilent Seahorse XF Cell Mito Stress Test of Naïve T cells (200 K/well) seeded in XFp PDL Miniplates (p/n 103722-100) in Seahorse XF RPMI medium, pH 7.4 supplemented with 10 mM glucose, 2 mM glutamine, and 1 mM pyruvate. The red box highlights measurements which are in the precision improvement range (≤40 pmol/min).

Intuitive and flexible: Access your XF data anywhere with Seahorse Analytics

To complete the workflow, analyze the XF HS Mini result data with Agilent Seahorse Analytics. Seahorse Analytics is a Mac- and PC-accessible web-based data analysis application that provides secure data storage, powerful and versatile data analytics and visualizations, and intuitive features for data management, export, and sharing, to simplify the most important step of the workflow—understanding your results.

The XF HS Mini Analyzer, with system improvements for measuring low respiratory rates (OCR) when paired with the XF Hu T Cell Activation Kit, XFp PDL Miniplates, and Seahorse Analytics is an excellent solution for analyzing low respiring T cells and many other cell types that exhibit a low dependency on aerobic respiration due to quiescence, inherent metabolic poise, or metabolic dysfunction.

Figure 4. Analyzing data using Agilent Seahorse Analytics. XF Cell Mito Stress Test of Naïve T cells (200 K/well) seeded in XFp PDL coated miniplates (p/n 103722-100). After three basal measurements, cells were treated with vehicle (Control) or CD3/CD28 antibodies (Activated).

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