

Technical Note: Scanning Seahorse Bioscience XF96 Cell Culture Microplates

Introduction

The Seahorse Bioscience XF96 Extracellular Flux Analyzer is an instrument that simultaneously measures two energy yielding pathways; aerobic respiration and glycolysis, in a microplate format. Like many instruments, cell culture quality control and confluence consistency are important considerations for obtaining reproducible results. In addition to the 24-well format, a new vessel definition has been created for the IncuCyte platform to allow imaging of Seahorse 96-well microplates in order to measure cell confluence. With this definition, Seahorse Bioscience XF96 V3-PET and V3-PS cell culture microplates were successfully imaged, but did present imaging challenges in the IncuCyte platform. The most significant challenge was the presence of three unavoidable raised features, or probe checks, on the bottom of each well that fall within the 10x field of view (FOV) (Figure 1). We also found that scratches and manufacturing

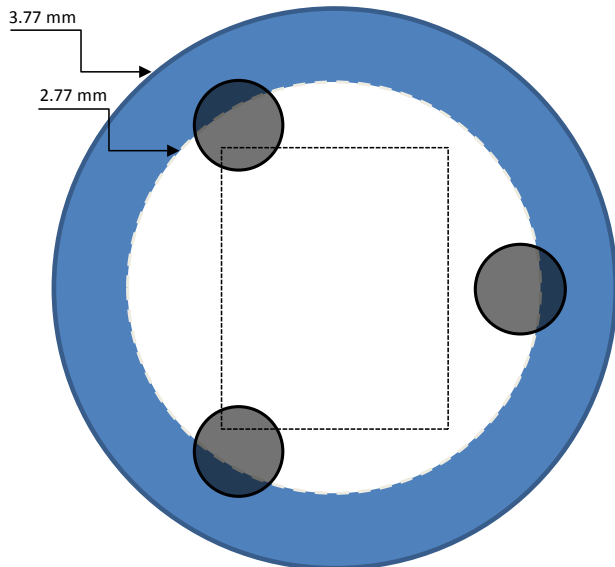


Figure 1: The dotted lined box in this image illustrates the 10x field of view within the imaging area (inside the blue ring) of one well of the Seahorse XF96 microplate. The probe checks (grey circles) are unavoidable for imaging in the IncuCyte FLR with a 10x objective.

artifacts within the microplate wells minimally affected confluence measurements yet did not alter cell growth.

Because of these challenges, the vessel definition found within the IncuCyte GUI for scanning Seahorse Bioscience XF96 cell culture microplates will remain unsupported. Essen BioScience will not provide technical support for mechanical problems, issues associated with focus, miscalculations of confluence, and other problems associated with scanning the Seahorse XF96 cell culture microplates. This document is intended to describe and inform users of these potential problem areas. The experiments described in this study were completed using an IncuCyte FLR equipped with a 10x objective. Both motion calibration and 384-well calibration were completed on the instrument within 60 days prior to completing these studies. The reduced surface area of the Seahorse XF96 cell culture microplates make it critically important that both calibration procedures are completed regularly (at least quarterly).

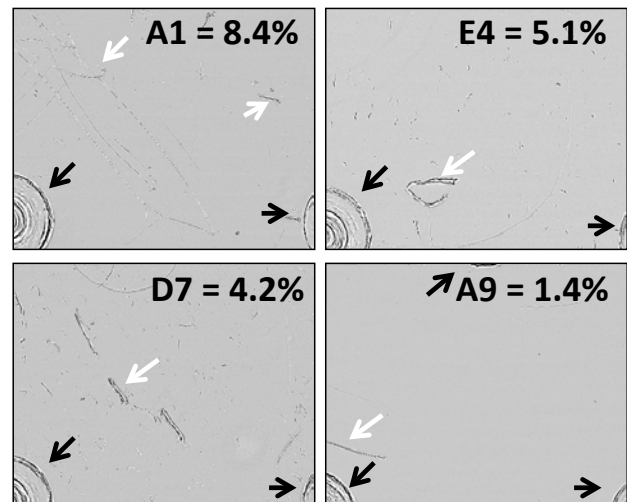


Figure 2: Each well in the Seahorse XF96 cell culture microplate was scanned in IncuCyte FLR. Probe checks (black arrowheads) and manufacturing artifacts (white arrowheads) were clearly visible in each well of the scanned plates. These artifacts contribute to the calculation of confluence in each well (presented as percentages).

Methods and Results

Probe checks and plastic imperfections affect confluence measurements in an empty plate

To determine the contribution of the probe checks and manufacturing artifacts in the plastic to confluence, an empty (containing no cells) XF96 cell culture microplate (V3-PET; Part No. 101104-004) was scanned three independent times, and confluence measurements were analyzed. As shown in Figure 2, in addition to the probe checks, numerous artifacts in the plastic including scratches and molding abnormalities were observed, but only slightly affected the confluence measurement. More accurately, the confluence values ranged from 1.4% to 8.4% with a mean confluence of 3.3% (Figure 3). Both the mean confluence and the range of confluence values were consistent from scan-to-scan with a mean well-to-well %CV over all 96-wells scanned equalling $8.3\% \pm 8.1\%$. Therefore, either the overall mean confluence of empty wells (3.3%) or the percent confluence on a per well basis could serve as a background number if the user thought it necessary to subtract the “background” confluence from values obtained from cells in culture.

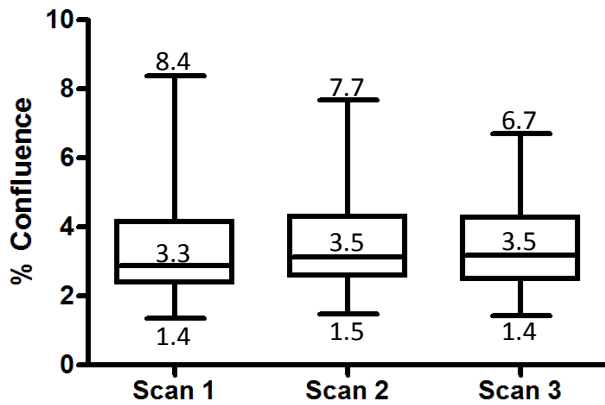


Figure 3: All 96 wells of an empty XF96 V3-PET cell culture microplate were scanned three independent times and confluence was recorded. This box-whisker plot illustrates the variation in the confluence numbers from the whole plate. The line in the middle of the box represents the mean. The whiskers represent the range of confluence values recorded.

Growth of MDA-MB-231 cells in XF96 cell culture microplates

In this experiment, decreasing concentrations of MDA-MB-231 human breast adenocarcinoma derived cells were plated in duplicate columns (N=16) of both the XF96 V3-PET (part No. 101104-004) and the XF96 V3-PS (part No. 101085-004)

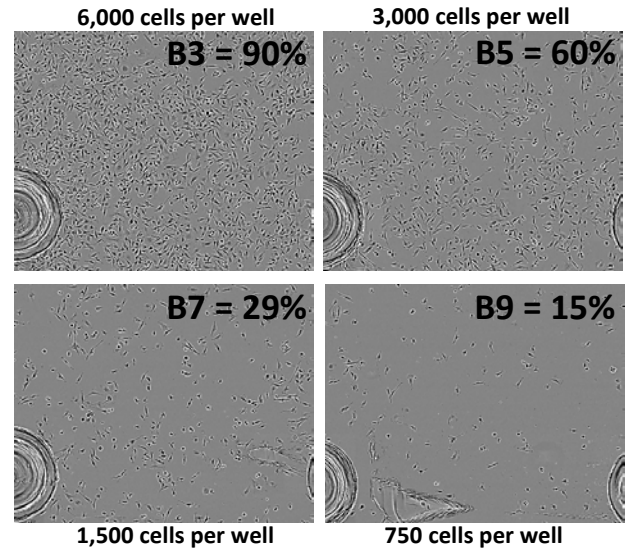


Figure 4: MDA-MB-231 cells were plated at the indicated densities and imaged every 2 hours in an IncuCyte FLR equipped with a 10x objective. These representative images were captured 18 hours post seeding. Both probe checks and manufacturing artifacts in the plastic are easily observable in each image.

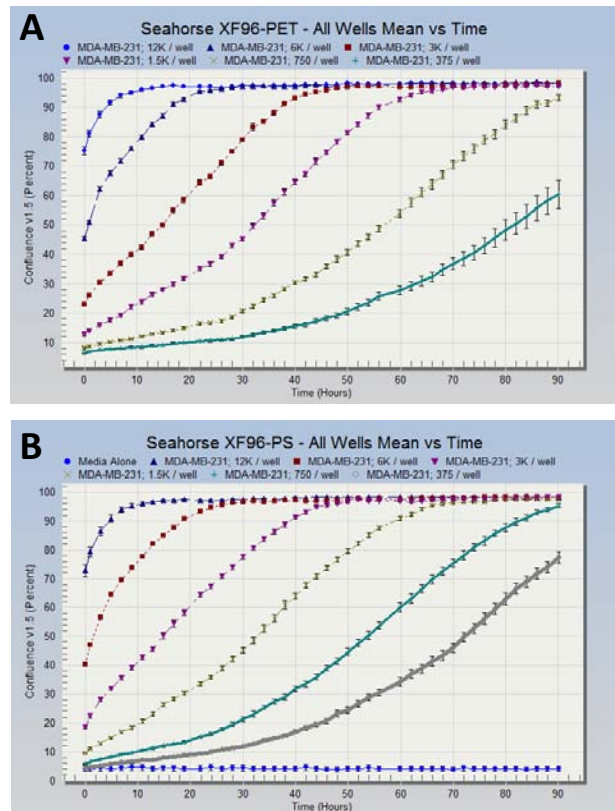


Figure 5: MDA-MB-231 cells were plated in both the XF96 V3-PET (A) and the XF96 V3-PS microplates (B) at the indicated densities and imaged every 2 hours in an IncuCyte FLR equipped with a 10x objective. Confluence was plotted over time. Data points represent mean \pm standard error. N=16 wells per density.

cell culture microplates. Phase contrast images were acquired at 2 hour intervals using an IncuCyte FLR equipped with a 10x objective. As shown in Figure 4, cell morphology was normal, and confluence values correlated well with seeding density 18 hours post plating. Furthermore, the IncuCyte FLR was able to accurately scan each well, calculate confluence, and generate kinetic growth curves of MDA-MB-231 cells similar to what we have observed using numerous other types of tissue culture plates (Figure 5A and B). Interestingly, we found that the growth rate of this cell type was nearly identical, with the exception of later time points at low seeding densities, in both the PET and PS microplates.

Conclusions and Recommendations

In general, the presence of the probe checks and plastic artifacts did not alter cell growth, and only minimally affected the measurements of confluence. Furthermore, using the “Seahorse XF96 V3-PET” vessel definition, we did not encounter a mechanical or focus failure in our instrument at Essen BioScience. For most downstream applications conducted using these plates, scanning the plate, and using the confluence numbers directly reported by the IncuCyte is most likely sufficient to properly quality control the assay. However, it is also possible that users may want to subtract the contribution of these artifacts before making an experimental decision. Based on the experiments presented in this report, we provide the following recommendations:

- 1) Perform 384-well and motion calibration procedures on the IncuCyte FLR prior to using the Seahorse XF96 cell culture microplates for the first time and repeat quarterly.
- 2) If following the recommended Seahorse cell plating procedure, i.e. if you are not plating cells into wells A1, A12, H1, H12, choose a scan pattern that **DOES NOT** include the scanning of these wells.
- 3) The values obtained with the cell type and conditions noted in this technical note yielded confluence calculations that ranged from 1.4% - 8.4% above the true confluence level. These variations will differ compared to the lot of

plates, cell type and growth conditions used in other experiments. Additional calculations might be necessary to compensate for these variations.

About the IncuCyte Live-Cell Imaging System

The Essen BioScience IncuCyte FLR Live-Cell Imaging System is a compact automated microscope. The IncuCyte FLR resides inside your standard tissue culture incubator and is used for long term kinetic imaging. To request more information about the IncuCyte please visit us at www.essenbioscience.com.