Simplifying Progress

Accelerating Cell Line Development with an Efficient, Combined Platform Approach

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Abstract

The determination of product critical quality attributes (CQAs), key process parameters and indicators (KPPs and KPIs respectively) is a critical step for process efficiency during cell line development. Thousands of clones are typically screened with the goal of finding those that are stable, produce high yields of the bioproduct and exhibit desired CQAs. Screening and process optimization activities are typically carried out at the small scale in bioreactor cultures and are aimed at ensuring that results translate to larger bioreactor scales. Performance monitoring is primarily based on cell growth, cell viability, metabolite analysis and product titer, and is assessed over the entire culture process duration.

Octet® BLI instruments utilize the biolayer interferometry (BLI) technology to monitor response changes upon the interaction of biological molecules. They can be easily implemented in cell line development labs to facilitate rapid determination of product yield amongst other attributes. Implementing state-of-the-art analytical technologies into the cell culture process development workflow can greatly reduce time to results. This capability allows informed decisions directly at the end of the process. It also enables subsequent experiments to be planned based on all of the performance data, rather than waiting days or weeks for product yield and other attributes data to be available. In this poster we show the power of using the Ambr® 15 Cell Culture system with an integrated Vi-CELL XR and Ambr® Analysis Module together with an Octet® R8 instrument to identify optimum yield conditions in media screening and process optimization experiments.

Figure 1: *Range of Octet® BLI Instruments Utilizing Label-Free and Fluidic-Free BLI Technology*

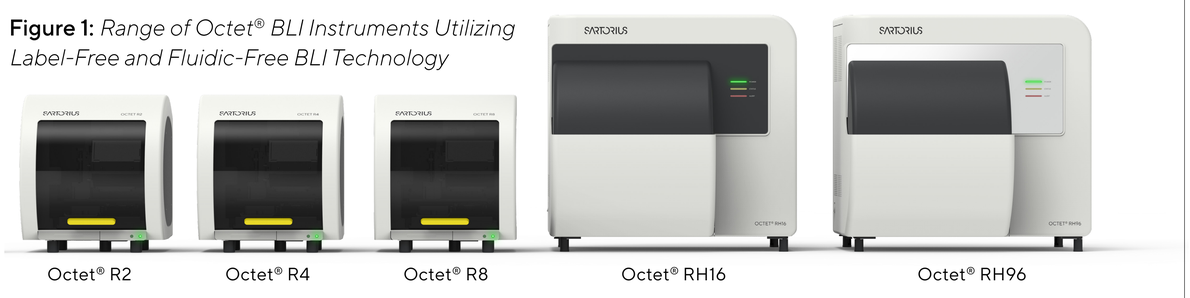
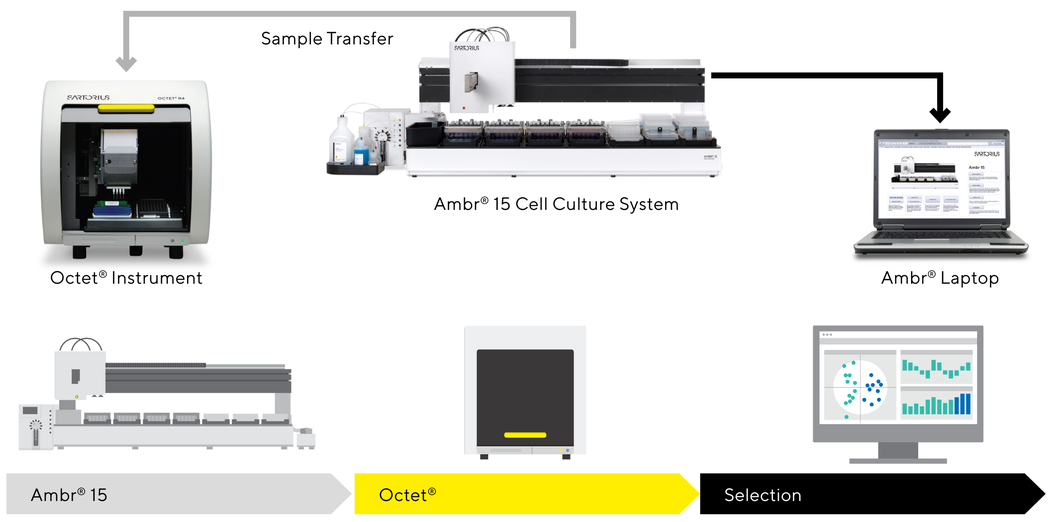


Figure 2: *The Octet® and Ambr® 15 Cell Culture Setup for Clone and Media SelectionTechnology*



Materials and Methods

Media Screening and Ambr® 15 Process Optimization

Ambr® 15 Cell Culture standard microbioreactors with sparge tube were inoculated with a density of 3E5 cells/mL at three different starting volumes ranging from 12 to 14 mL. Each microbioreactor has individual gassing via sparge tube into the culture. The pH and DO control loops maintained target set-points by applying CO2 and O2 as required, along with a fixed ballast gas (air). In this experiment the ballast gas (air) flow rates were between 0.05 to 0.25 mL/min. The process set points were as follows; pH 7.0 (upper limit of 7.1), DO 40% and temperature 36.8 °C.

Four different stirring speeds were investigated as part of the DOE experiment, these ranged from 1050 to 1650 rpm . Five different media and two different clones were investigated.

Media and Reagent Additions

Proprietary basal and feed media were used for the experiments. Daily feed and antifoam solutions were added to each vessel automatically for both experiments.

Sampling and Analysis

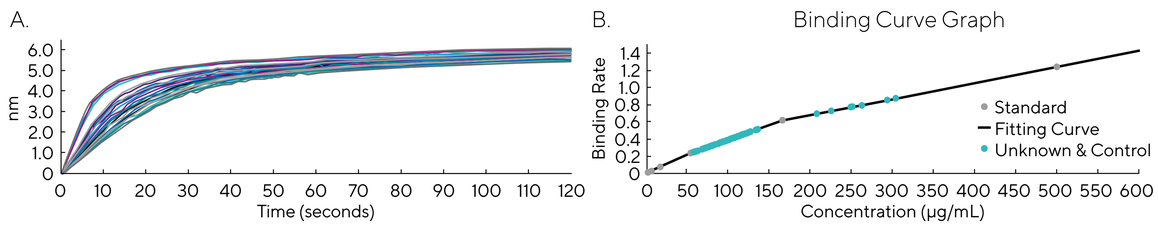
Viable cell concentration (VCC) counts were obtained via a coupled Vi-CELL XR (Beckman Coulter, USA). pH measurements were obtained via an integrated Ambr® Analysis Module. Glucose and lactate measurements were performed off-line with an EKF BIOSEN S-Line device (EKF-Diagnostic GmbH, Germany) according to manufacturer's protocol. Cell viability and cell count were determined daily with a cut-off for titer measurement set at 70% viability i.e. when the viability fell below 70%, titer was not measured. For titer measurement daily samples were taken automatically by the Ambr® 15 Cell Culture. These samples were centrifuged for 5 min at 6600 xg, the cell-free supernatant was removed and retained for titer measurement.

Titer Measurement in Octet®

The cell-free supernatant samples were diluted in the required 96-well plate. The plate was then vortexed for at least 1 minute at a moderate level. An Octet® QKe (older version of the Octet R8 system) was used with Protein-A biosensors. Protein A biosensors are best used for measurement of IgG concentrations between 1 and 500 µg/mL; for higher IgG titers the samples were diluted in media. Calibrator samples with known concentration were used for the generation of the standard curve. Biosensors were regenerated for re-use by dipping into regeneration buffer followed by neutralization buffer for 5 seconds each. Three cycles of regeneration were used. Quantitation assay time was set to 120 seconds with the sample plate temperature set to 30 °C.

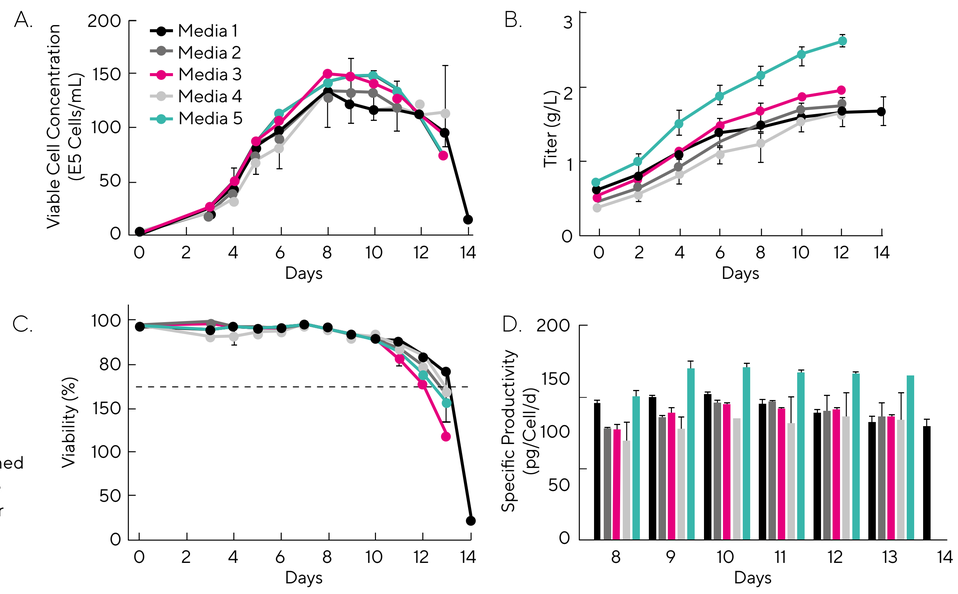
Results

Figure 3: *IgG Titer Measurement Using Protein A Biosensors*



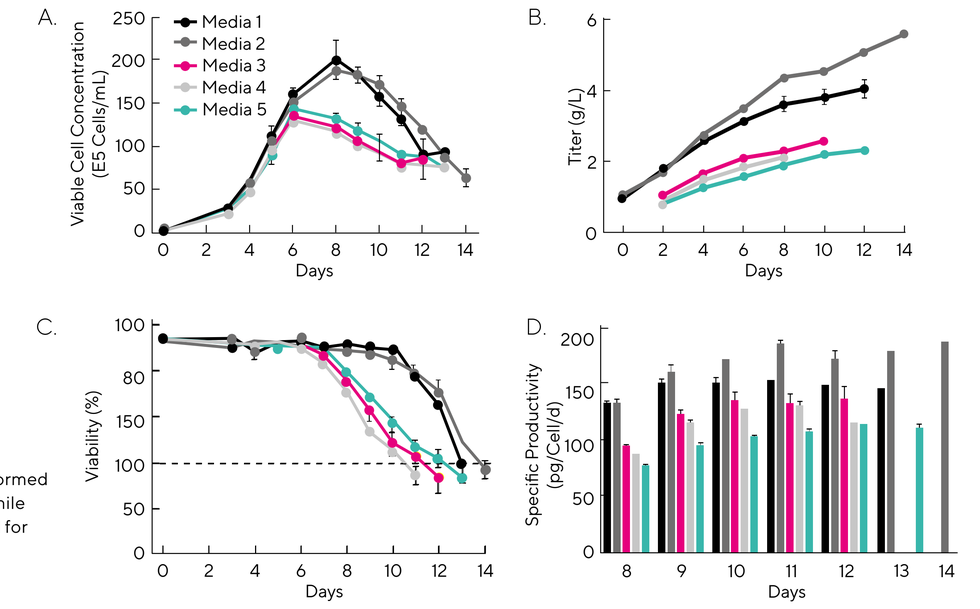
Raw data (A) represents two replicates each of the 5 media growth conditions for each clone. (B) All 96 Sample IgG Concentrations (Orange) Plotted on the Calibrator Curve (Blue, 0-500 (µg/mL).

Figure 4: *Time-Course Cell Count and Titer Analysis for Clone 1 in Different Media Types*



Cell Count was performed using Vi-CELL XR while the Octet® was used for titer determination.

Figure 5: *Time-Course Cell Count and Titer Analysis for Clone 2 in Different Media Types*



Cell Count was performed using Vi-CELL XR while the Octet® was used for titer determination.

Conclusion

The integration of the Octet® BLI platform and the Vi-CELL XR with the Ambr® 15 Cell Culture system within the cell line development workflow enables the data acquisition of multiple CQAs in one setup. When combined with process optimization DOE and the high-throughput capabilities of the Octet® BLI platform this workflow should allow cell line developers to rapidly optimize the clone and media selection process.

While the Octet® BLI instrument portfolio ranges from a 2-channel (two measurements simultaneously) to an 96-channel instrument (analyzing a maximum of 96 samples simultaneously), with varying throughputs, the Ambr® 15 Cell Culture automated microbioreactor system is able to run up to 48 × 15 mL cultures per experiment. When integrated, the two systems offer considerable advantages for screening of multiple cell lines or clones in parallel, and for reducing the experimental costs associated with media and feeds, especially for fed-batch or intensified bioreactor processes. Compared to traditional titer determination techniques such as ELISA or HPLC, the Octet® BLI approach enables a faster turnaround of yield analysis with minimal analyst involvement.

Reference

Sartorius Application Note: Cell Line Development: Accelerating Process Optimization by Combining Ambr® 15 Cell Culture with Octet® Titer Measurements