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Life Sciences

Cultivation of Mammalian Cells in the Cydem VT System Bioreactor Module

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Introduction

The importance of consistent clone screening is invaluable for cell line development and allows top clones to be confidently selected. Manual selection processes such as delivering multiple feeds and additives to individual shaker flasks by pipetting, sampling and delivering to standalone analytical devices, and compiling data from multiple devices are all prone to human error. In addition, manual technique may vary from operator to operator, which may contribute to variability in results. The Cydem VT Automated Clone Screening System automates these processes and allows for precise and repeatable clone selection. As an evolution of the BioLector bioreactor, the Cydem VT bioreactor module features fine-tuned delivery of O_2, CO_2 and N_2 gases via micro-channeled chips to individual wells, which creates a stable environment for mammalian cell growth and antibody production. This is done with proportional-integral-derivative (PID) controlled feedback looping using real-time pH and DO data from calibrated optical sensors attached to the bottom of the microbioreactors. The sensors are read with the integrated fluorescent LED detection system. The shaking and temperature-controlled incubation chamber with a sliding cover and fans is also a feature that ensures uniform shaking conditions and heating distribution. Scheduled feeds, base additions, and analytical tests also contribute to repeatable results by ensuring consistent nutrient delivery and testing times.

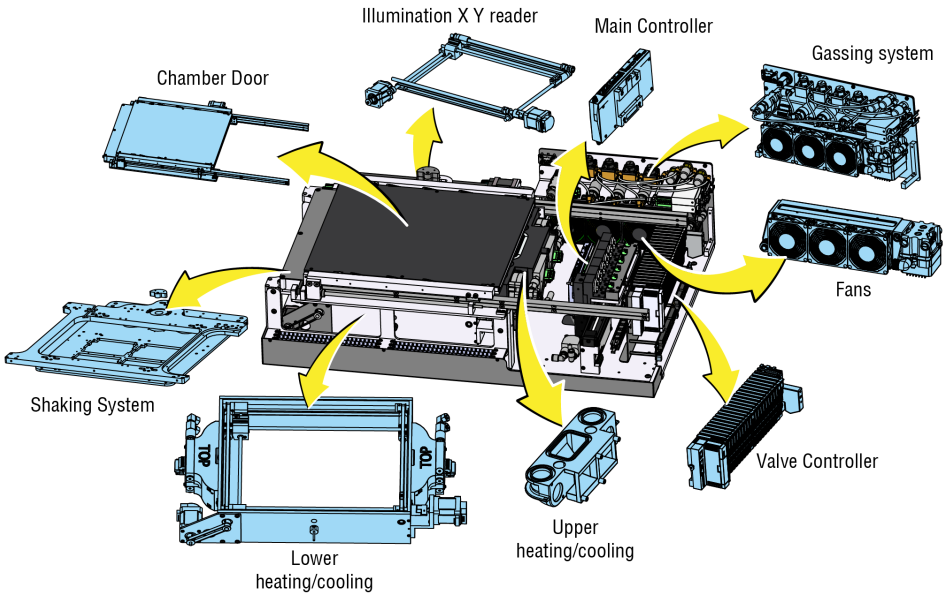

Figure 1: Cydem VT Bioreactor module. The bioreactor module provides uniform shaking and temperature conditions. The optical detection system provides in-process monitoring of pH and dissolved oxygen (DO) which triggers N_2, O_2 and CO_2 gases to be delivered to individual wells via feedback loops.

Cell Health Uniformity Experiment

To demonstrate uniformity over the 96 parallel microbioreactor wells of the Cydem VT system, CHO-K1 cultures were sampled on various days and analyzed on the Cydem VT cell health analysis module for three 7-8 day fed-batch culture experiments. 50 mL bioreactor culture tubes were run in a shaking Eppendorf incubator with similar conditions as controls for each experiment. Base additions were not performed for culture tubes. A single clone of the CHO-K1 cell line was used for all experiments.

Cydem VT Culture Conditions

A user-defined, Cydem VT system protocol with conditions of a 50% DO set point, 36.5°C and 800 rpm shaking speed was created. CO_2 and/or N_2 was flushed into the wells to lower or raise pH to a gassing adjusted setpoint of pH 7.0. The base addition target point was at pH 7.1, and base addition trigger point at pH 6.8. pH was regulated by the PID controlled feedback loops.

CHO-K1 cells were cultured in ActiPro liquid medium supplemented with 6 mM Glutamine and 375 µg/mL geneticin. On day 0, the initial stock culture was grown to approximately 4.0 E+06 viable cells per mL and diluted in fresh media to a cell density of 0.5 E+06 viable cells per mL in a final working stock volume of 500 mL. The initial stock culture concentrations and viability were determined using a Vi-CELL BLU cell viability analyzer. Bioreactor plates were manually seeded by pipetting 5 mL of cell suspension into each well. The process was repeated until all 4 bioreactor plates were seeded. Media-specific pH and DO optode calibration values were used. As a control, 10 bioreactor tubes with 5 mL of 0.5 viable cells per mL were incubated in an Eppendorf incubator with 5% CO_2 and 36.5 °C temperature at 250 rpm.

Feed and additives

The experiment was set up to have Feed A and Feed B scheduled to be added daily on days 3-7 at 3% and 0.3%, respectively. 32 µL of 400 g/L glucose was added to each well on day 6 based on a previously determined feeding strategy of feeding to 5 g/L when values drop below 3.5 g/L. The media specific scale factor was set to 180* and represents the amount in µL of 6% Na_2CO_3 to be added to increase 10 mL of culture media by 1 pH unit. Base was added 2 times per day on a per well basis when the pH reached a value below the 0.1 unit set point. Feeds and glucose were placed on deck prior to their first use. Na_2CO_3 was placed on deck at the beginning of the experiment. The 50 mL bioreactor control tube cultures were fed manually with a pipette.

***Note:** Scaling factor values should be determined and verified for specific experimental conditions.

Cydem VT Cell Health Analysis

The technology of the cell health module is based on the Vi-CELL BLU cell viability analyzer and delivers the same consistent results. For cell health analysis, the Cydem VT system samples 43 µL from each microbioreactor using stainless steel fixed tips and delivers the sample to the cell health module at the scheduled times. The default mammalian cell type settings were used for analysis.

Cell Health Results

Uniformity was evaluated by examining the coefficients of variation percentages (CV%) of viable cell density (VCD) measurements for the microbioreactor replicates across all days for each experiment (Figure 1). A variability plot of individual data points for day 7 Cydem VT system cell health data was compared to day 7 bioreactor tube controls analyzed with the Vi-CELL BLU cell viability analyzer (Figure 2 and Table 1). Mean CV% of viable cell density across all days was 5.47% (N=576), 4.33% (N=576), and 4.99% (N=672) for experiments 1, 2, and 3, respectively (Figure 1). On any given day for all experiments, CV% did not exceed 7.5% (data not shown). Sampling days varied for each experiment with days 1,2,3,4,7,8; 1,2,4,5,6,7; and 1,2,3,4,5,6,7 for experiments 1, 2 and 3 respectively. 96 replicates were measured on the Cydem VT system and 10 replicates for the 50 mL bioreactor tube controls per day for each experiment. Data was analyzed using JMP version 16.1.0.

Mean CV% Across All Days by Experiment

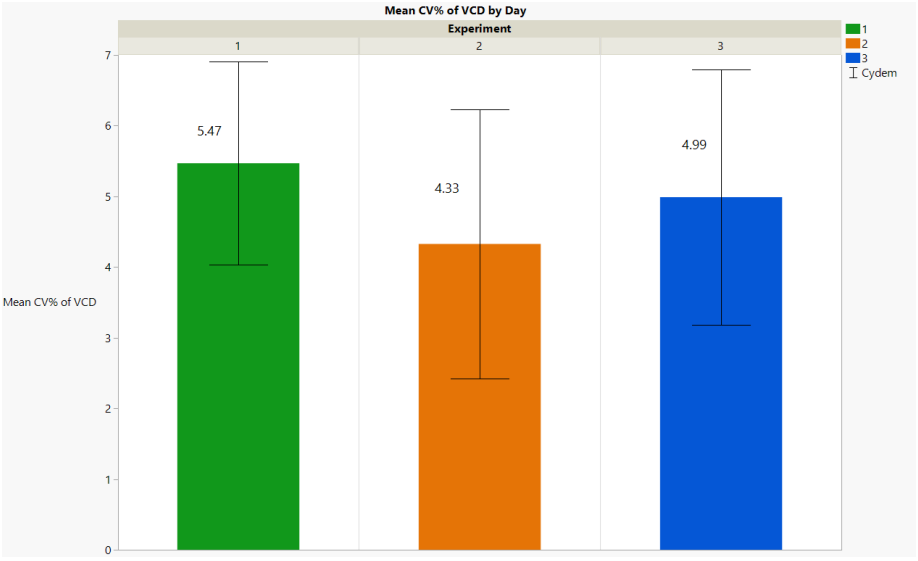

Figure 2: Mean CV% of VCD was calculated by taking the average CV% across all days for each Cydem VT Automated Clone Screening System experiment. Error bars are ±1 standard deviation (SD) from the mean. CV% did not exceed 7.5% on any day for any experiment. Sampling days varied for each experiment with days 1,2,3,4,7,8; 1,2,4,5,6,7; and 1,2,3,4,5,6,7 for experiments 1, 2 and 3 respectively (N=576, 576, and 672 replicates for experiments 1, 2 and 3, respectively).

Cell Health Day 7 Variability

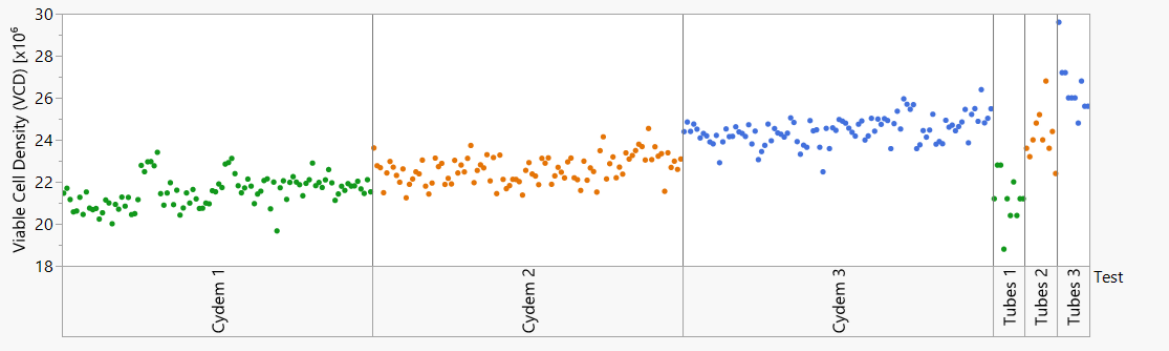

Figure 3: Day 7 variability chart for 3 experiments on the Cydem VT system and with 50 mL bioreactor tubes in a shaking Eppendorf incubator. 1, 2, and 3 refer to experiments 1, 2, and 3, respectively.

Test	VCD Mean [x10^6 cells/mL]	Std Dev	CV%	VCD Range [x10^6 cells/mL]	Replicates
Cydem 1	21.54	0.73	3.41	3.74	96
Cydem 2	22.60	0.66	2.93	3.30	96
Cydem 3	24.45	0.65	2.65	3.91	96
Tubes 1	21.20	1.19	5.63	4.00	10
Tubes 2	24.20	1.21	5.00	4.40	10
Tubes 3	26.48	1.33	5.03	4.80	10

Table 1: Day 7 VCD Mean, standard deviation, CV%, and VCD Range for 3 experiments run on the Cydem VT system and for 50 mL bioreactor tube controls.

Conclusion:

The Cydem VT Automated Clone Screening System consistently demonstrated a uniform growth pattern, with a mean coefficient of variation (CV%) of VCD below 5.5% across all days for all three 7-8 day fed-batch experiments. Moreover, the CV% of VCD on day 7 was lower in the Cydem VT system compared to the 50 mL bioreactor tube controls in all experiments. This can be attributed to the system's temperature uniformity, constant shaking speed, and feedback loop mediated gas regulation, which ensured consistent and repeatable growth conditions across multiple experiments. Overall, the Cydem VT system consistently delivers reliable and predictable cell health results for week-long fed-batch experiments.

Product not for use in diagnostic or therapeutic procedures.

Product in development. Performance characteristics have not been validated.

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