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White Paper

Use of a Chemically Defined Cloning Medium for Quality and Regulatory Risk Mitigation in Cell Line Development

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Successful cell line development establishes the foundation for a robust manufacturing process and is critical for reducing regulatory risk and ensuring quality. Isolation of a stable, high-performing clone is also essential for optimizing process economics by delivering the desired titer and growth rate, enabling a scalable process, and accelerating time to market. Importantly, generation of a clonally derived population of cells for production of a biotherapeutic is needed to maintain critical quality attributes (CQAs) such as post-translational modifications which can affect the safety and efficacy of the biotherapeutic. While clonally derived and non-clonally derived cell banks may initially deliver the same CQA mean value, use of a non-clonal population can lead to variability of these attributes, have higher susceptibility to shifts, and be subjected to unforeseen selective pressures during the manufacturing lifetime.[1]

A specially formulated cloning medium is typically used during cell line development to support growth and viability during selection and cloning processes, which are performed at low cell densities and put a great deal of stress on the cells. This whitepaper describes the performance and stability of a novel, chemically defined cloning medium for use in cell line development. The specially formulated medium accelerates clone isolation, reduces the risk of variability in protein quality, reduces product and process impurities, and ensures a consistent performance. Because it is chemically defined, regulatory risk is reduced as is the potential for a disruption in supply.

The Cell Line Development Process

Figure 1 provides a simplified view of the cell line development process based on the CHOZN® GS$^{-/-}$ expression platform. A monoclonal antibody (mAb) expression vector is first introduced into the CHOZN® GS$^{-/-}$ cell line via electroporation. Transfected cells are plated at a low density of 5,000 cells per well in 96-well plates in the absence of glutamine. Cells that do not take up the expression vector will not be able to produce glutamine and will not survive. This part of the cell line development process is also stressful for the cells that have taken up the mAb expression vector; these cells must make enough glutamine synthetase from the vector to survive without glutamine in the medium. Of the thousands of cells plated per well, many will not be viable; over time, their contents will be released into the wells of the 96-well plate, causing further stress on the remaining, viable cells.

The resulting minipools of cells are then scaled up and screened to identify the highest producers, from which single cell clones will be isolated. The cells may produce different titers and have different growth profiles and quality attribute profiles.

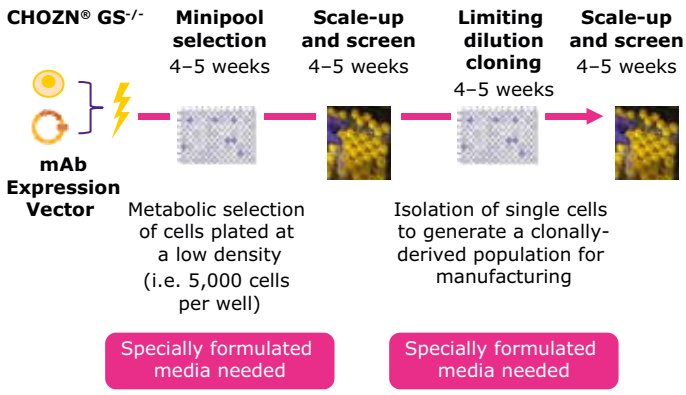

Figure 1.

The cell line development process is stressful for cells and requires specially formulated media.

Limiting dilution is the most accessible technique for isolating clones. The cell population is diluted to a very low density and distributed among the wells in a 96-well plate at a density of half a cell per well, with a goal of isolating single cells in individual wells. This low density is stressful for the cells, and many do not survive. Given these challenging conditions and the low efficiency of the process, use of a robust medium formulation is essential for isolating as many clones as possible and ensuring high clone recovery.

Performance of Cellvento® 4CHO-C Cloning Medium

Cellvento® 4CHO-C Cloning Medium is a chemically defined formulation designed for use in cell line development. It can be used to replace the current EX-CELL® CHO Cloning Medium which contains hydrolysates and is not chemically defined.

The performance of this medium was evaluated in minipool selection and limiting dilution cloning processes. In the first study, minipools of CHOZN® GS$^{-/-}$ cells producing a mAb, CHOZN® GS$^{-/-}$ parental cells, and a CHO-K1 cell line producing alkaline phosphatase were plated at half a cell per well in Cellvento® 4CHO-C Cloning Medium and imaged on day seven to quantify outgrowth. The results were normalized to cells plated in non-chemically defined EX-CELL® CHO Cloning Medium containing hydrolysates (Figure 2). Results demonstrated robust clone outgrowth in wells plated in Cellvento® 4CHO-C Cloning Medium, across the three different cell lines. The relatively lower outgrowth observed for the CHO-K1 cells was likely due to the cloning process which is known to be highly variable.

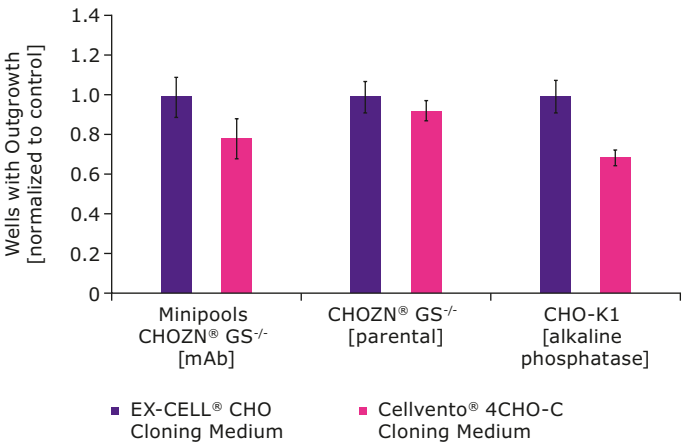

Figure 2.

Comparison of outgrowth in cell lines grown in non-chemically defined (purple) or chemically defined (pink) cloning media.

Subcloning of four stable CHOZN® GS$^{-/-}$ cells production clones in the same conditions as the above study showed that the outgrowth is reproducible and similar to that achieved using the non-chemically defined cloning medium (Figure 3).

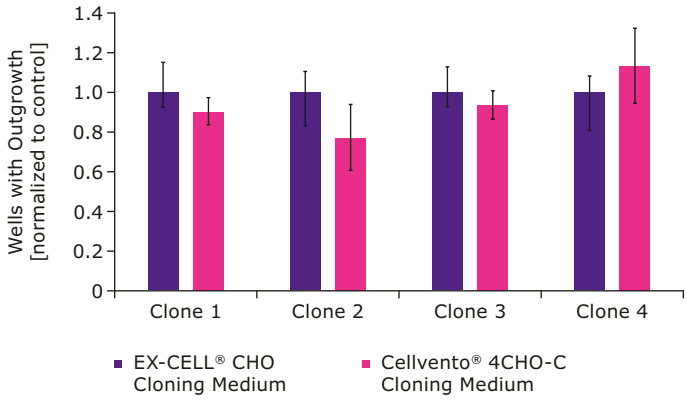

Figure 3.

Comparison of outgrowth in four subclones grown in non-chemically defined (purple) or chemically defined (pink) cloning media.

In a subsequent study, the impact of using a combination of chemically defined cloning medium and condition medium on clone outgrowth was evaluated. Conditioned medium is commonly used in the cloning process to improve outgrowth; it is thought to contain beneficial growth factors and other components secreted by healthy cell cultures that help cells survive low-density conditions. Conditioned media was generated by first inoculating CHOZN® GS$^{-/-}$ cells from a high-viability culture grown in EX-CELL® CD CHO Fusion medium in mid-logarithmic growth phase at 1×10^6 viable cells/mL. Cells were incubated for 24 hours and sampled to ensure high viability and an appropriate doubling time. After which, the culture was clarified by centrifugation followed by sterile filtration of the clarified culture to obtain the conditioned medium.

Cellvento® 4CHO-C Cloning Medium was supplemented with conditioned medium at 20% of the total plating volume. The same experimental design was used as described above. Cells were plated in 96-well plates at half a cell per well, imaged on day seven of culture to quantify outgrowth and normalized to control (EX-CELL® CHO Cloning Medium).

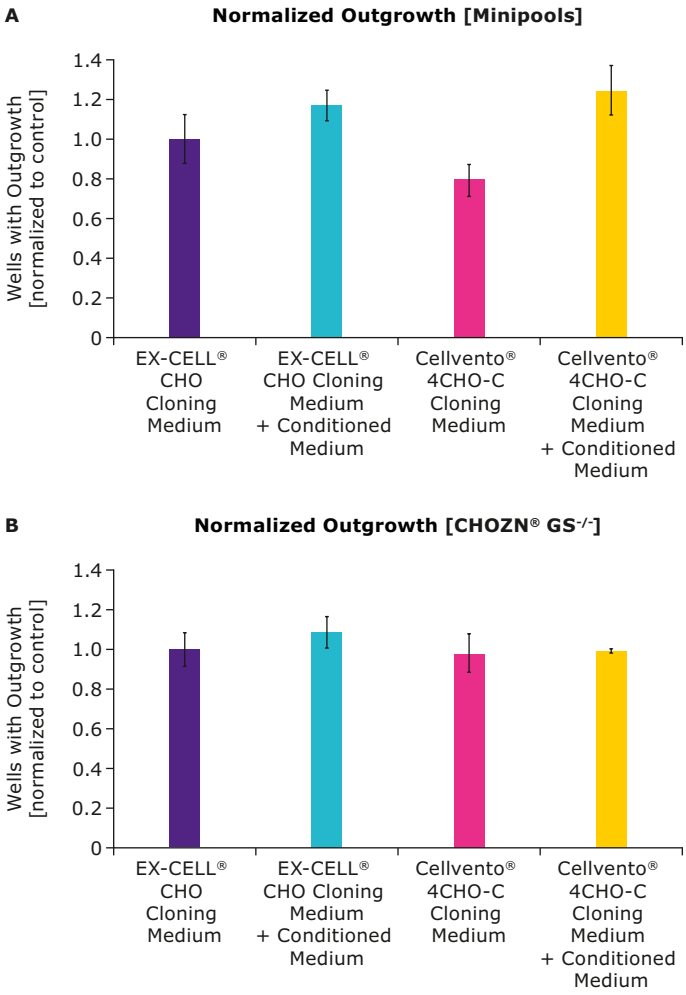

Figure 4.

Minipool (A) and parental cell line (B) outgrowth in non-chemically defined and chemically defined cloning media supplemented with conditioned medium.

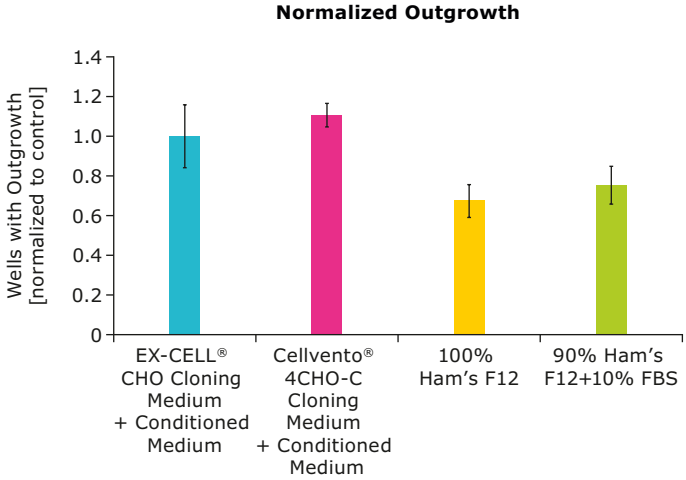

Figure 5.

Comparison of outgrowth in cloning media compared to Ham's F12 Medium with or without FBS.

Figure 4 shows the outgrowth of minipools (A) from CHOZN® GS$^{-/-}$ cells producing a mAb or the parental cell line (B) in either non-chemically defined or chemically defined cloning medium, with or without addition of conditioned medium. In both cases, addition of conditioned medium boosted cloning performance, yielding more clones for screening and supporting their viability in low density conditions.

Figure 5 provides a comparison of outgrowth when clones were grown in either one of the cloning media or in 100% Ham's F12 with or without supplementation of 10% fetal bovine serum (FBS) which are the historical industry benchmarks for cloning medium. Both the non-chemically defined and chemically defined media outperformed Ham's F12 with or without FBS.

While the cloning step is much more sensitive and requires specialized cloning medium, Cellvento® 4CHO-C Cloning Medium can also be used to support minipool generation which also subjects cells to stressful selection and low-density conditions. In the CHOZN® GS$^{-/-}$ expression platform, the goal of the minipool step is to create heterogeneous populations that enhance the probability of finding high producing, stable clones. This is accomplished by plating cells at a low density of approximately 5,000 cells per well in the selection medium which contains no additional glutamine, 24–48 hours after transfection. Over the course of one to two weeks, cells that express enough glutamine synthetase to survive will proliferate, fill the well, and become a full colony; these colonies are then screened to assess productivity.

Figure 6 shows the mAb titers produced by minipools grown in 96-well plates in either the non-chemically defined or chemically defined cloning media (note that low titers are normal at this stage of cell line development). While there is one outlier, which is not surprising, no significant differences were observed between the two media.

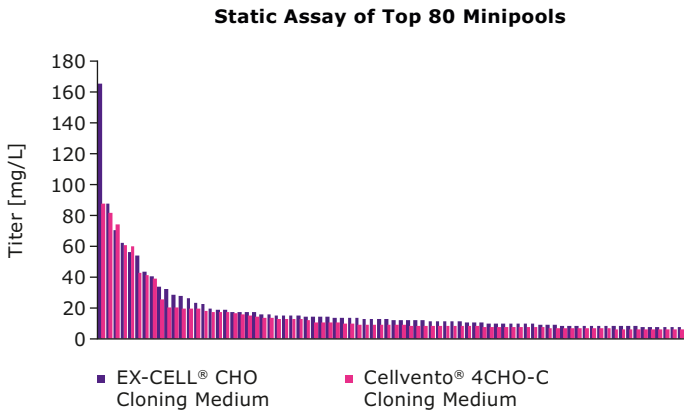

Figure 6.

Comparison of titers from 80 minipools grown in non-chemically defined (purple) or chemically defined (pink) cloning media.

Stability of Cellvento® 4CHO-C Cloning Medium

A critical aspect of cell culture media is its stability over time. To examine the stability of Cellvento® 4CHO-C Cloning Medium, the outgrowth of clones cultured in fresh medium or medium stored for either six or twelve months at 2–8 °C was compared. As shown in Figure 7, all media performed at an equivalent level and was comparable to a fresh bottle of the non-chemically defined cloning medium.

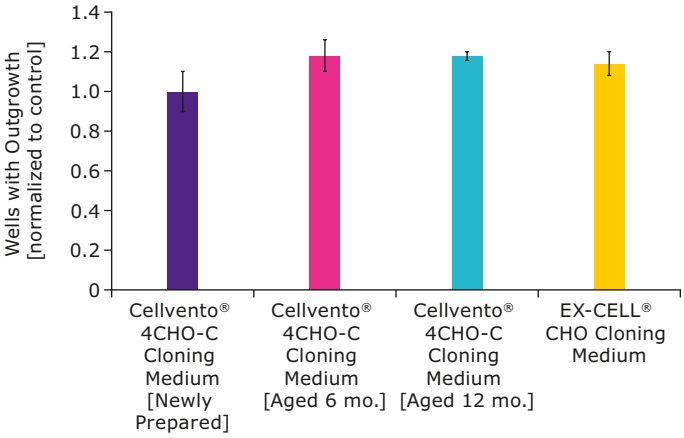

Figure 7.

Comparison of chemically defined cloning medium stored for up to one year with fresh, non-chemically defined cloning medium.

Figure 8 shows the pH profile of the chemically defined cloning medium (purple), as compared to the non-chemically defined medium (yellow) over the course of 56 days when exposed to ambient air at 2–8 °C. The increase in pH of the Cellvento® 4CHO-C Cloning Medium was much smaller compared to the non-chemically defined cloning medium indicating that an entire bottle of cloning medium can be used without concerns over performance over time.

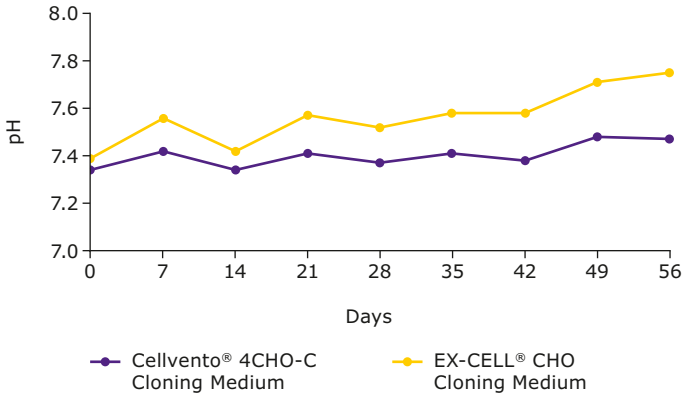

Figure 8.

pH profile of non-chemically defined (yellow) or chemically defined (purple) cloning media exposed to ambient air for 56 days.

Conclusion

Selection of a high producing, stable clone sets the stage for cost-effective drug manufacturing. As part of the cell line development process, transfected cells are grown at very low densities and subjected to culture conditions that are less than optimal in order to apply the appropriate selective pressure. Given these conditions, the right cloning medium helps ensure optimized cell growth and viability during the selection and cloning process, and the ability to achieve clonality.

As described above, chemically defined Cellvento® 4CHO-C Cloning Medium supports robust minipool and clone outgrowth at a level comparable to our EX-CELL® CHO Cloning Medium which is not chemically defined. The Cellvento® 4CHO-C Cloning Medium offers the ability to isolate higher rates of clones and minipools, accelerating the cell line development workflow. Chemically defined media offers superior consistency between lots and a reduced risk of variability in protein quality which translates into reduced regulatory risk. Greater lot-to-lot consistency also improves process robustness, delivering a more consistent performance across multiple cell line development processes.

Reference

1. Welch JT, Arden NS. Considering “clonality”: A regulatory perspective on the importance of the clonal derivation of mammalian cell banks in biopharmaceutical development. Biologicals. 2019 Nov; 62:16-21. doi: 10.1016/j.biologicals.2019.09.006. Epub 2019 Oct 3. PMID: 31588011.

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