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Application Note

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Enhancing Media Screening: Proven Performance With Pre-Selected Media and Feed Combinations for CHO Cell Culture Optimization

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Abstract

Selecting the right cell culture medium and feed is crucial for achieving high protein titers and the desired critical quality attributes. The complexity of screening multiple media and feed options can be effectively managed using Ambr® 15 bioreactors—often with a single feed strategy. This approach enables the selection of the best media and feed combination based on small-scale performance. Accompanying analytics (such as amino acid analysis) enable efficient process optimization, including feed regime adjustments or custom feed formulations.

In this study, various proprietary CHO cell culture media and feed solutions were tested under controlled conditions in Ambr® 15 bioreactors, using a single feed strategy and commercially available CHO cell lines. The optimal media-feed combinations and conditions were successfully scaled up to benchtop bioreactors. While the 4Cell® SmartCHO System demonstrated high performance in Sartorius CHO DG44 cells, 4Cell® CHO Medium TCX6D and 4Cell® CHOlean Medium were more suitable for the growth and performance of CHO-K1 and CHO-S cell lines. Finally, 4Cell® CHO-GS TCX10D Medium was specifically designed for, and performed optimally with, CHO-GS cell lines. Across all media and feed combinations, 4Cell® SmartCHO feeds supported high productivity of all CHO cell lines tested.

Introduction

Various media formulation strategies have been used to cultivate cells in vitro for the high-level expression of protein-based therapies. Bioprocesses employed in recombinant protein production are under strict regulatory control for safety purposes, with the use of animal-derived components in the media representing the biggest concern. Bioprocesses using Sartorius media will avoid these regulatory hurdles with our chemically defined and animal component-free formulations.

The Sartorius media portfolio supports the cultivation of any CHO cells— the most widely used cell type in the production of biomolecules. Our CHO media are designed and optimized to support the high-density suspension culture of a variety of CHO cell subtypes. Each medium and its corresponding feeds have been designed for use with a specific CHO cell expression system in suspension culture but may also be suitable for other CHO cell types.

While media screening can be time-consuming, identifying the right formulation and feed is essential to promote robust growth performance and high product yields. Our comprehensive CHO media portfolio can accelerate media development and increase the chances of finding the best media for each unique application. We recommend that users perform a benchmark study to identify the most compatible media and feed for their cell line and application. Our experienced media development team is available to support and enhance these evaluations.

Performance

4Cell® SmartCHO: Advanced, High-Performing CHO Cell Culture Media for CHO DG44, CHO-K1 and CHO-S

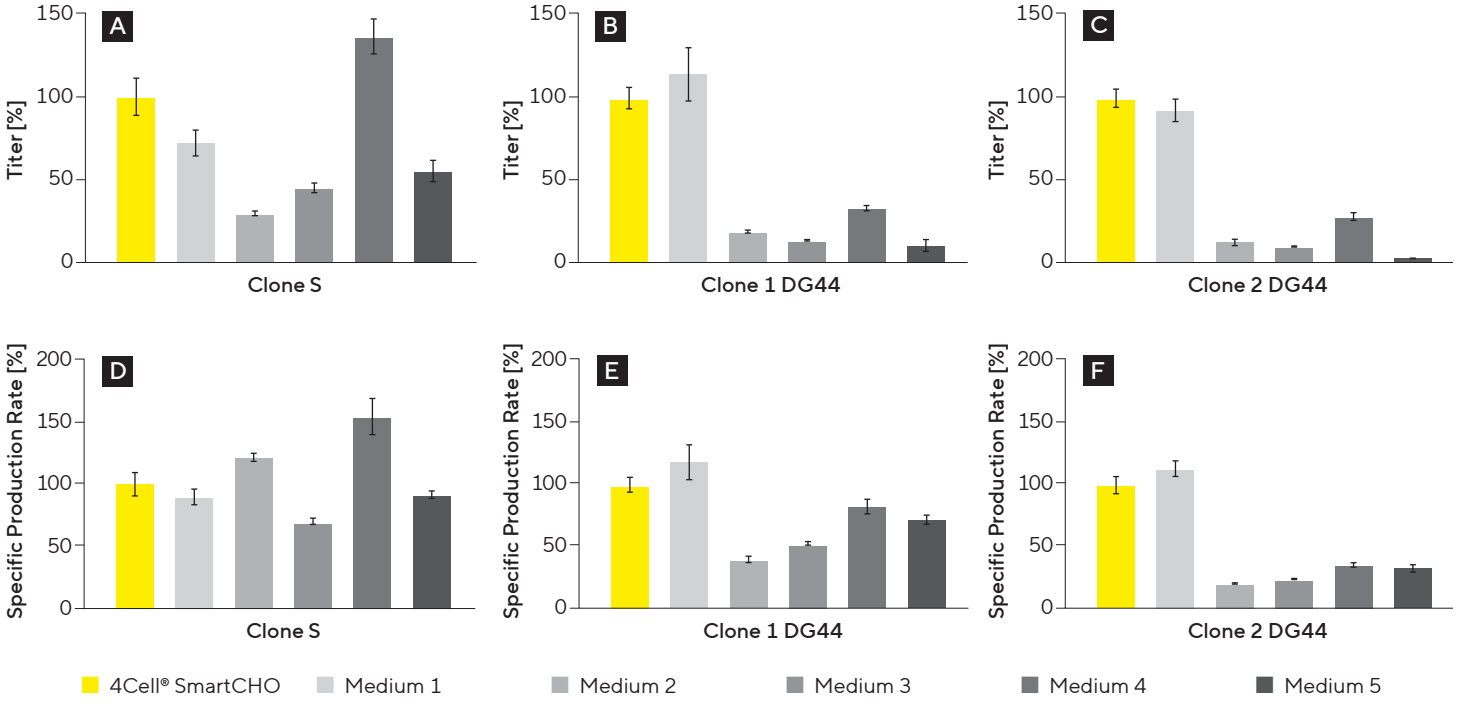
1.1 Supporting Fed-Batch Processes

With demonstrated robust performance in fed-batch applications and ease of transfer to perfusion processes, 4Cell® SmartCHO Production Medium with 4Cell® SmartCHO Feed Medium A (FMA) and Feed Medium B (FMB) — i.e., the 4Cell® SmartCHO system — is the first choice medium for CHO DG44, CHO-K1, and CHO-S cell lines, delivering robust growth and productivity. Although developed with a focus on CHO DG44 cells, the basal media and feeds have been tested and found to support growth across a broad range of CHO cell lines and product formats, including monoclonal antibodies (mAbs), bi-specifics, and Fc-fusion proteins.

We conducted a benchmarking study to evaluate the fed-batch culture performance of the 4Cell® SmartCHO system compared to selected commercially available media (Figure 1) using CHO-DG44 cell lines. In addition, we also tested its performance with CHO-S, CHO-K1, and CHO-GS clones, all cultured in the Ambr® 15.

Our findings show that the 4Cell® SmartCHO system supports the robust growth of CHO clones, performing as well as or better than commercially available media (Figure 1). The specific productivity of CHO cells varied significantly across clones and media, highlighting the need to screen multiple formulations, feeds, and parameters depending on the cell line and application (Figure 1).

Figure 1: *Titer and Specific Productivity of CHO Clones Cultured in the 4Cell® SmartCHO System vs. Commercially Available Media*

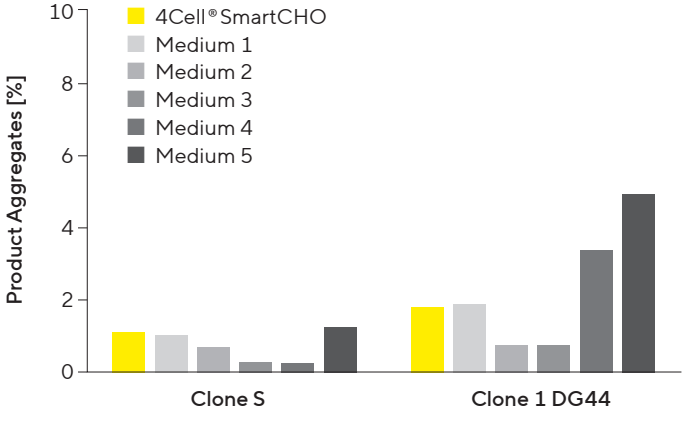


Note. Titer [%] **(A-C)** and specific productivity rate [%] for CHO-S **(A and D)**, Clone 1 CHO-DG44 **(B and E)**, and Clone 2 CHO DG44 **(C and F)** cells cultured in 4Cell ®SmartCHO and five commercial media.

Cell culture media have a significant impact on product quality attributes (PQAs). Size-exclusion chromatography reveals that the CHO-S clone maintains low aggregate values independent of the media, with some small variation (Figure 2). For CHO-DG44 (Clone 1), the 4Cell® SmartCHO system supported low aggregation levels (Figure 2).

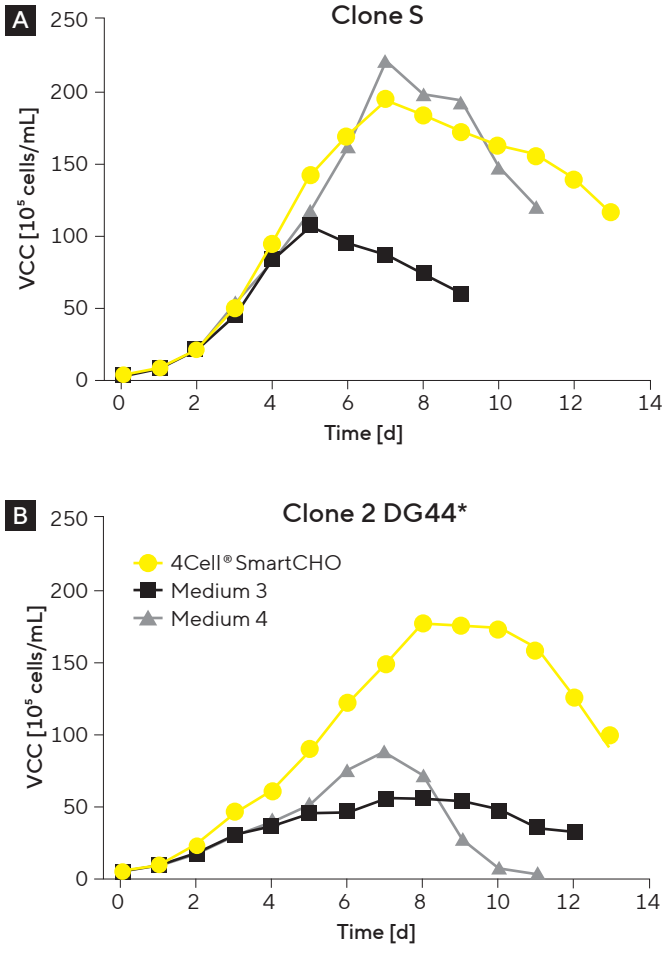
Cell culture media performance must be consistent across scales. The 4Cell® SmartCHO system shows robust and scalable growth performance when cultivations are carried out in a 5 L bioreactor (Figure 3).

Figure 2: *Product Aggregation in CHO Cells Cultured in the 4Cell® SmartCHO System vs. Commercially Available Media*



Note. Product aggregation determined by size exclusion chromatography.

Figure 3: *The 4Cell® SmartCHO System Supports Robust Growth of Two CHO Clones in 5 L Bioreactor Cultures*

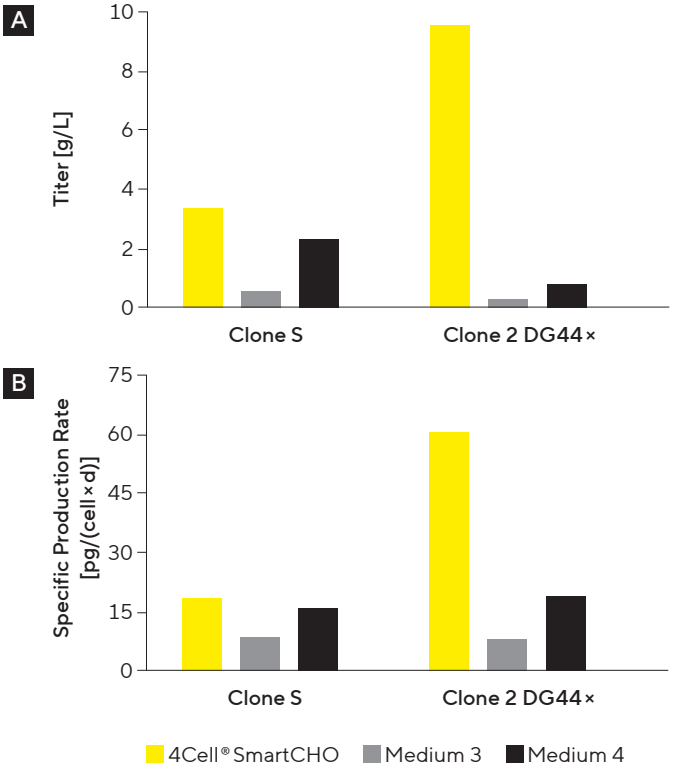


Note. CHO-S **(A)** and CHO-DG44 cells. Cells were adapted to the media for four weeks before testing. VCC = viable cell count. Clone performance with Media 4 was underestimated due to partial culture overfoaming.

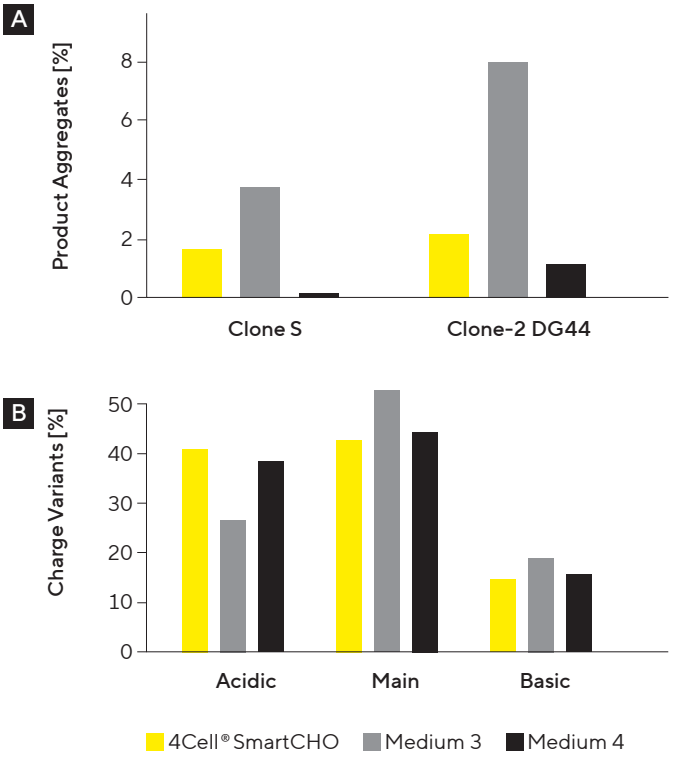
The 4Cell® SmartCHO system can support the production of high titers with high cell-specific productivity and lower aggregate formation (Figure 4) in large-scale cultures. This behavior was confirmed for different product classes (IgG1 and IgG4, data not shown).

In order to evaluate the performance of the 4Cell® SmartCHO system in different cell lines, a blind study was carried out by an external partner using a CHO-K1 cell line. The study showed that the 4Cell® SmartCHO system achieved the highest titer—surpassing even the performance of clone-specific, customized media (Figure 5).

Figure 4: *Productivity of and Product Quality Attributes of CHO-S and CHO-DG44 Cultures Grown in the 4Cell® SmartCHO System vs. Commercial Media*

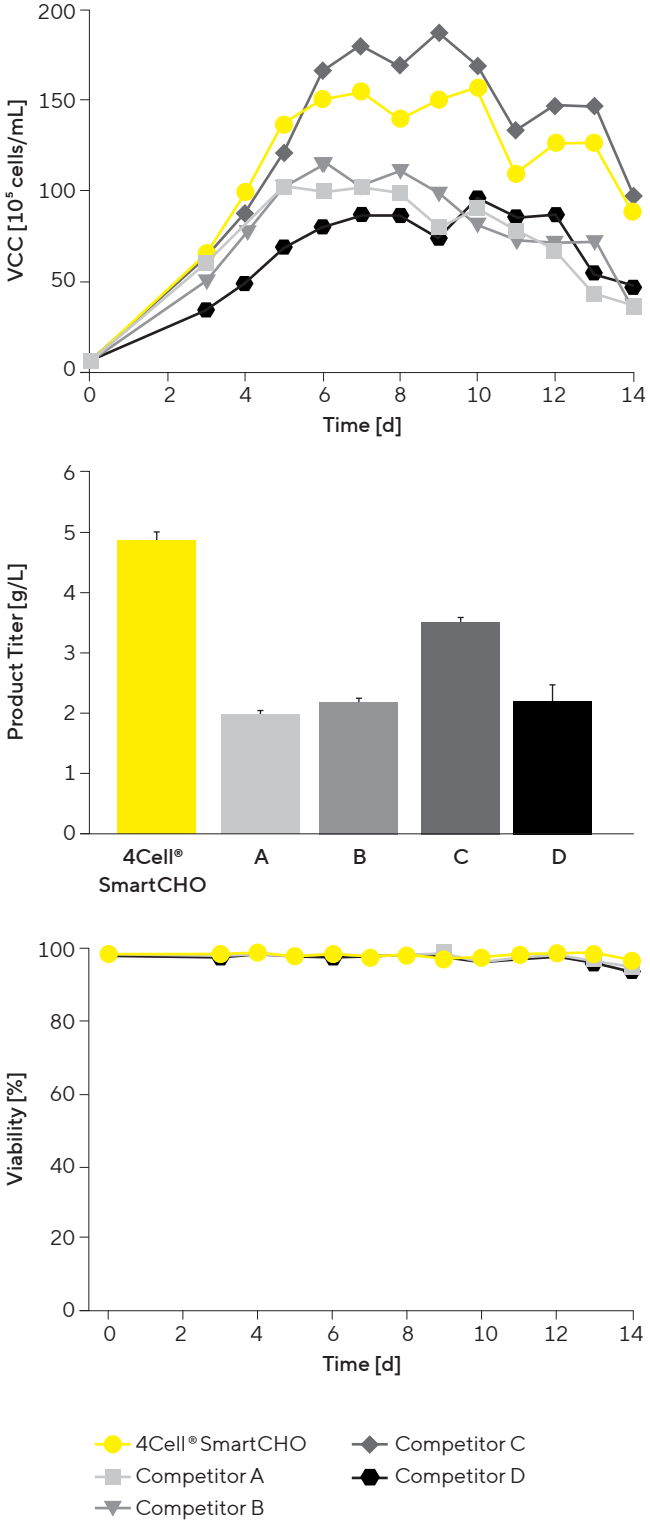


Note. **(A)** titer and **(B)** specific productivity.



Note. Aggregates **(A)**, charge variants for CHO-S cells only **(B).**

Figure 5: *Viable Cell Concentration (VCC), Cell Viability, and Product Titers of CHO-K1 Cells Cultured in Fed-Batch Mode Using the 4Cell® SmartCHO Cell Culture System vs. Commercially Available Media*



1.2 Supporting Intensified Processing

The 4Cell® SmartCHO system also supports protein intensification strategies. Three CHO-DG44 clones were tested in fed-batch and high-inoculation fed-batch (HIFB) processes (Figure 6). The results showed that the 4Cell® SmartCHO system could support high titers in both standard and high inoculation fed-batch processes. This demonstrates the suitability of the cell culture media for intensified manufacturing strategies.

The 4Cell® SmartCHO system can support process intensification by N-1 perfusion (Figure 7). To demonstrate this, we cultured CHO cells in high-density perfusion cell cultures (100 million cells/mL) for 5 – 7 days. These cultures were then used to populate a 200 L bioreactor in standard fed-batch mode, showing comparable growth and viability to expansion in a standard seed train (Figure 7).

Figure 6: *Productivity of the 4Cell® SmartCHO System Across Three CHO-DG44 Clones in Fed-Batch and HIFB Processes*

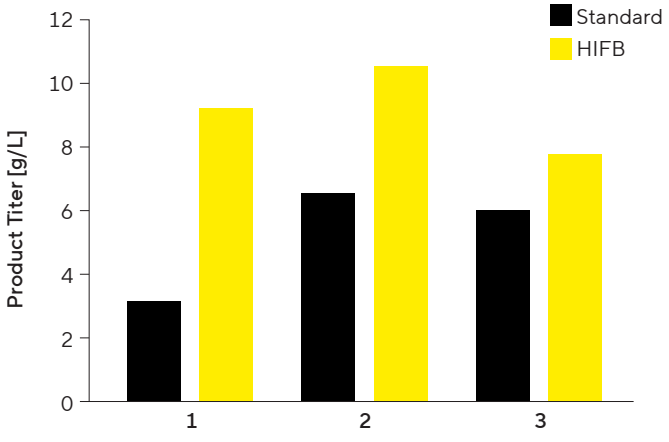
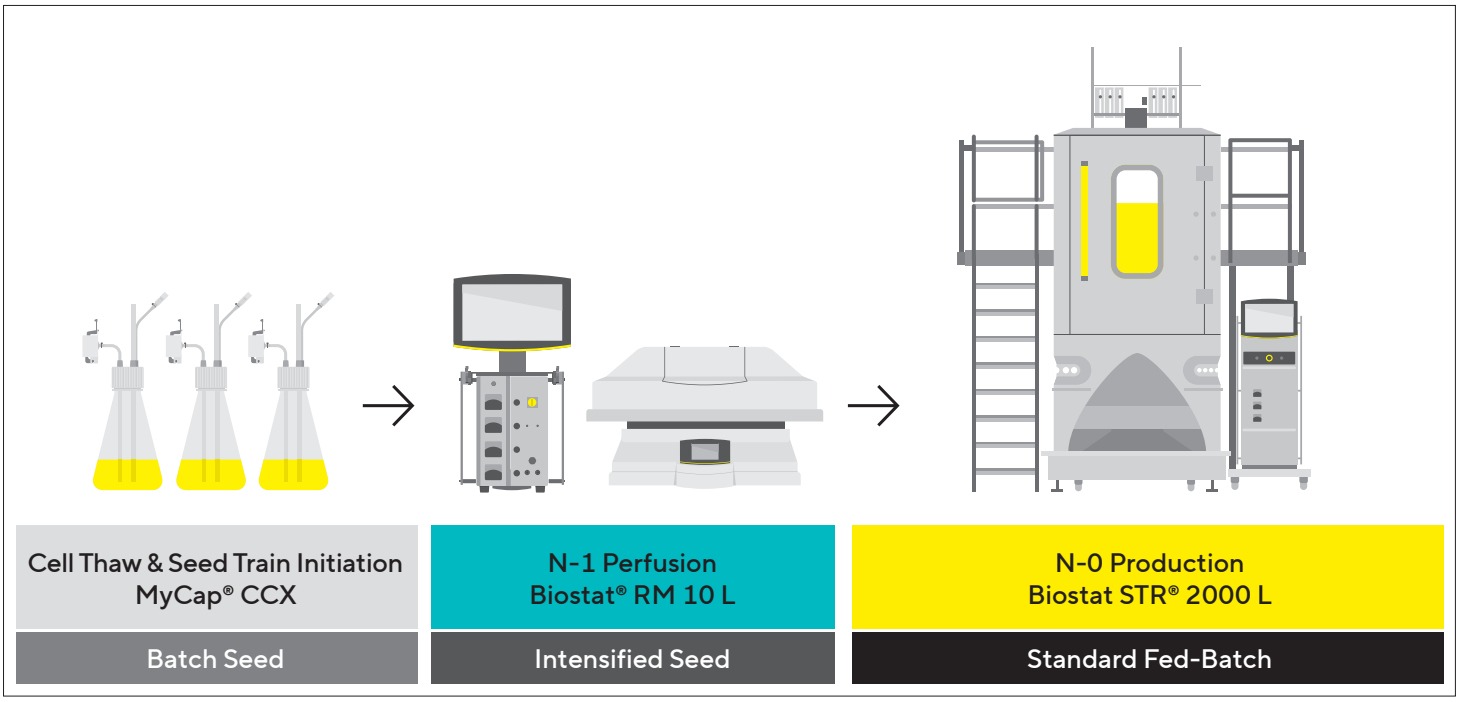
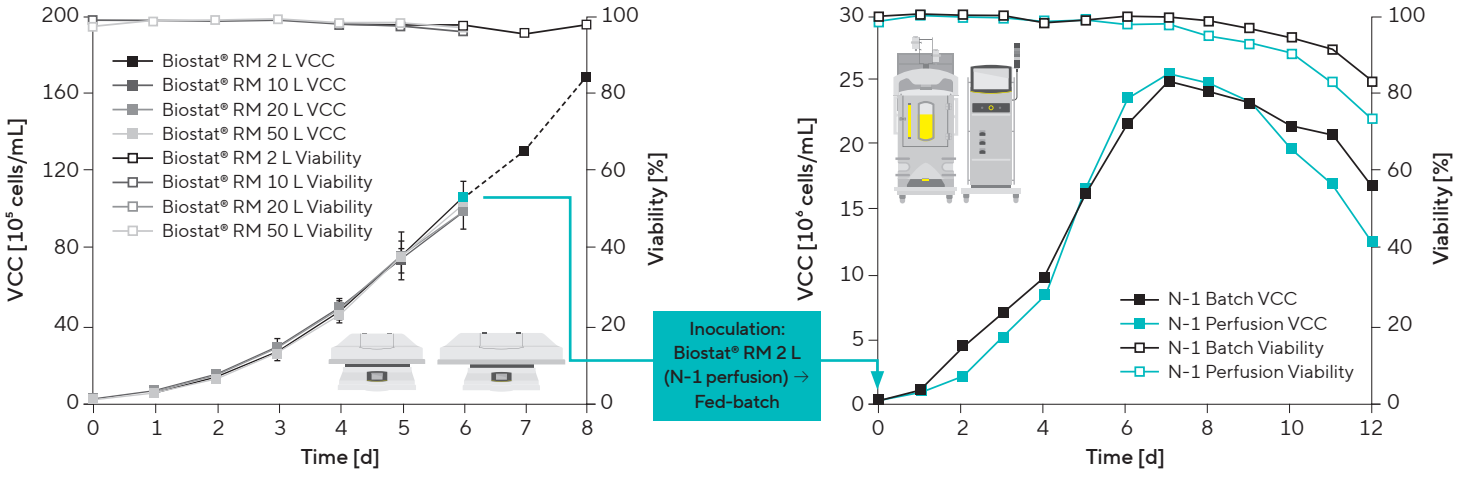


Figure 7: *Performance of the 4Cell® SmartCHO System in an N-1 Process*





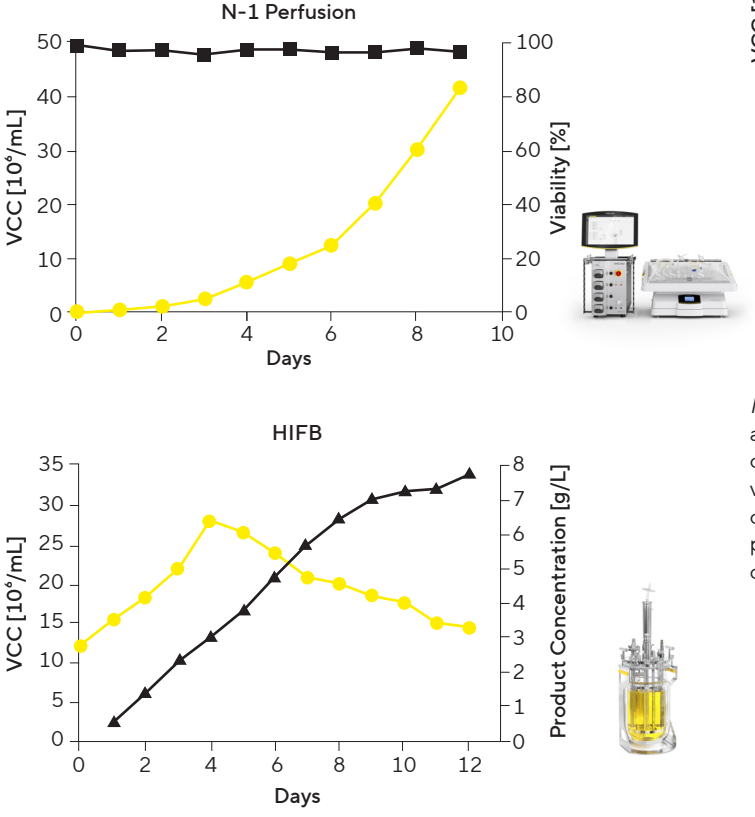
Note. Seed train intensification by N-1 Perfusion **(A)**, growth performance of CHO cells cultured in 4Cell® SmartCHO across different seed train scenarios **(B)**, growth Performance of CHO cells cultured in 4Cell® SmartCHO in a Biostat STR® 200 L production bioreactor **(C)**.

Using HIFB enabled by **N-1 perfusion** can help to increase productivity in the 10 g/L range, depending on the CHO clone (Figure 8).

The N-1 HIFB strategy with the 4Cell® SmartCHO system accelerates CHO-DG44 process development by enabling faster transition to the production phase and reducing lag time.

This approach is especially beneficial with the 4Cell® SmartCHO system, as it provides consistent conditions to evaluate growth, viability, and protein yield. It supports quicker optimization and improved scalability, making it ideal for high-efficiency mAb production.

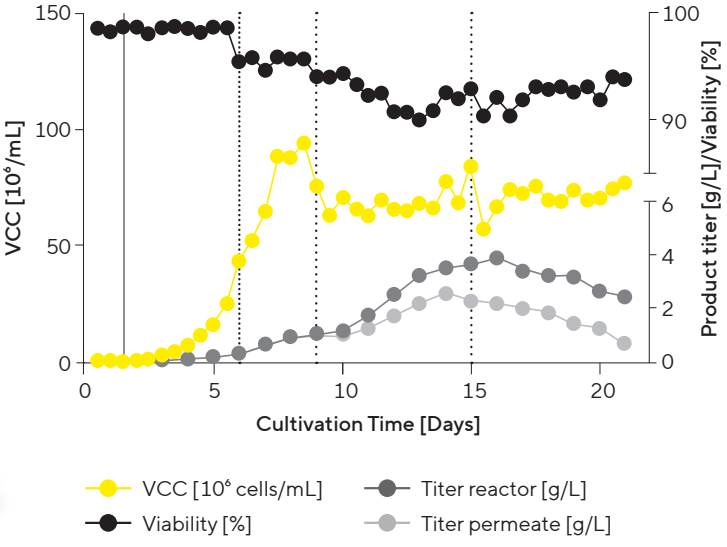
Figure 8: *Performance of the 4Cell® SmartCHO System in an N-1 Perfusion Process Prior to HIFB Culture*



Note. N-1 perfusion: cultivation was performed in the Biostat® RM System with 4Cell® SmartCHO at 1 L working volume. HIFB cultivation was carried out in 5 L Univessel® with 4Cell® SmartCHO as basal medium, and 1.3×4Cell® SmartCHO feeds added to provide adequate nutrient supply throughout the whole process. VCC = viable cell concentration.

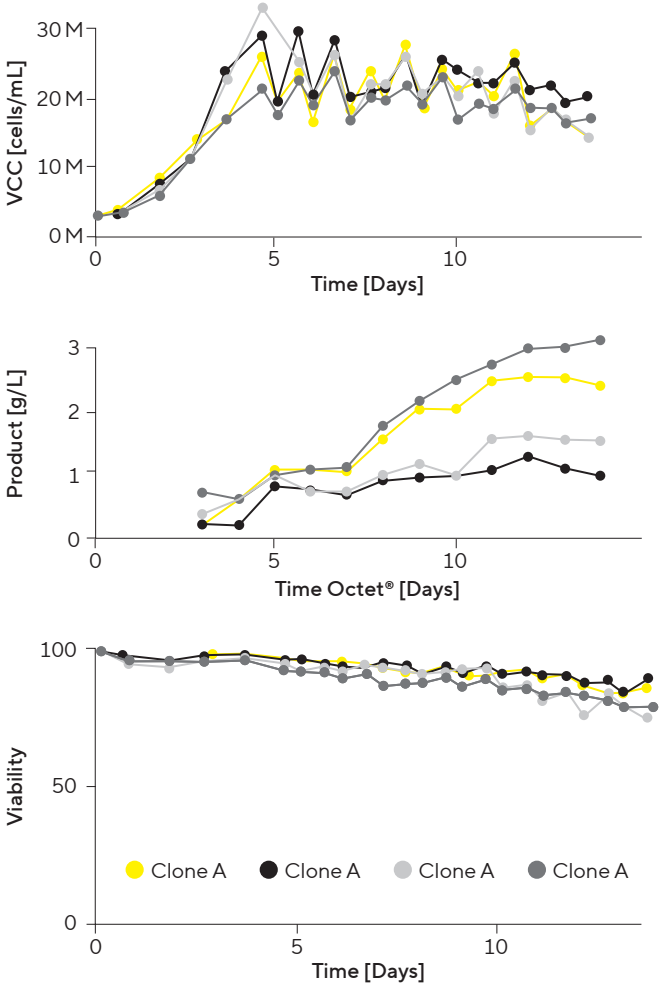
The 4Cell® SmartCHO system also supports **continuous perfusion** applications. Figure 9 shows data from a study in which a 2 L bioreactor with an alternating tangential flow (ATF) device was used to grow cells in the 4Cell® SmartCHO system in continuous perfusion. Cells reached and maintained a high cell density, viability, and productivity, and importantly, the process was successfully run for 21 days.

Figure 9: *4Cell® SmartCHO System Performance in Continuous Perfusion Cultures.*



Note. The continuous perfusion process was successfully conducted for a duration of at least 21 days in Ambr® 250 ATF, reaching a cell density of 70 million cells/mL. Utilizing the 4Cell® SmartCHO system with a volume-to-volume dilution of 1.25, the process maintained a cell viability of over 90%. Additionally, the process achieved a maximum volumetric productivity of greater than 2 g/L/day in the permeate. VCC = viable cell concentration.

Figure 10: *The 4Cell® SmartCHO System Performs Well in a Perfusion Mimic Process*

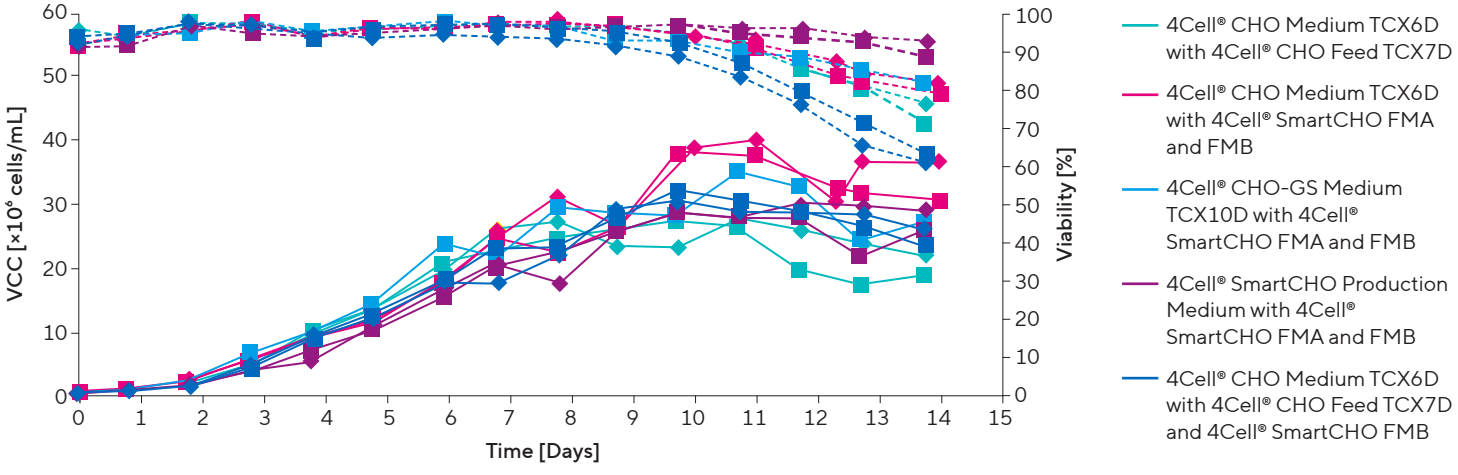


The perfusion mimic protocol used in our CHO Cell Line Development Service with the 4Cell® SmartCHO system in Ambr®15 has proven to be suitable for clone selection in perfusion applications (Figure 10). This approach effectively discriminates against unwanted clones, leading to the selection of the top-performing clone for perfusion processes.

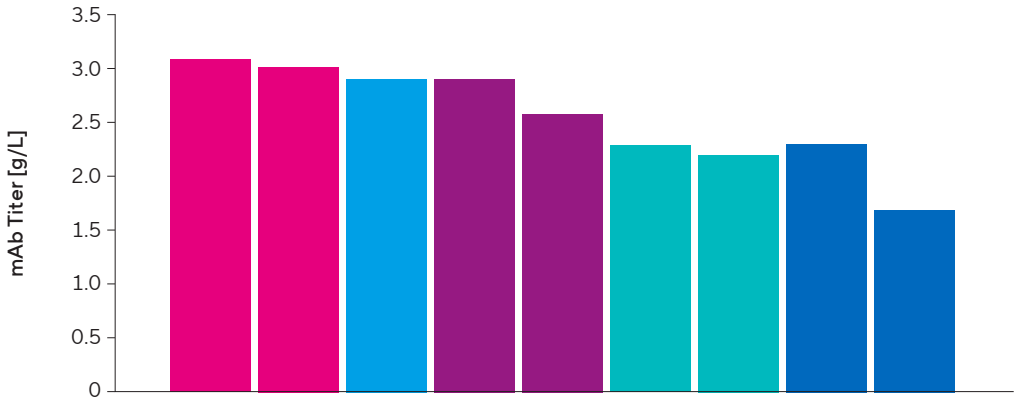
4Cell® CHO Medium TCX6D and 4Cell® CHOlean Medium for CHO-K1 and CHO-S Cell Lines

To assess various media and feed combinations across different cell lines, CHO-K1 cells were cultivated in Ambr® 15 fed-batch processes. CHO-K1 cells were cultivated in Ambr® 15 fed-batch processes. Glucose was used as the lead substrate to determine the feed regime. By combining 4Cell® CHO Medium TCX6D with 4Cell® SmartCHO FMA and FMB, an average titer of over 3.06 g/L was achieved (Figure 11).

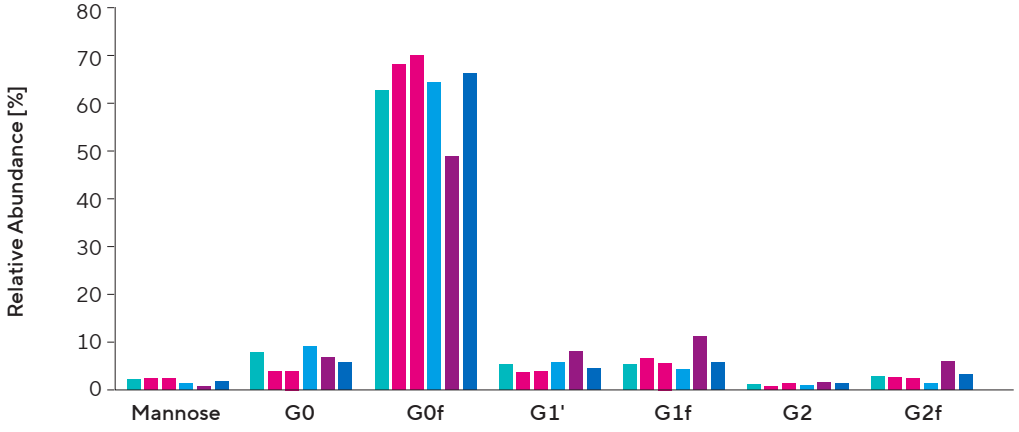
Figure 11: *CHO-K1 Viable Cell Concentration (VCC) and Viability, Antibody Titers, Glycosylation Profiles, and Charge Variants With Different Media | Feed Combinations*



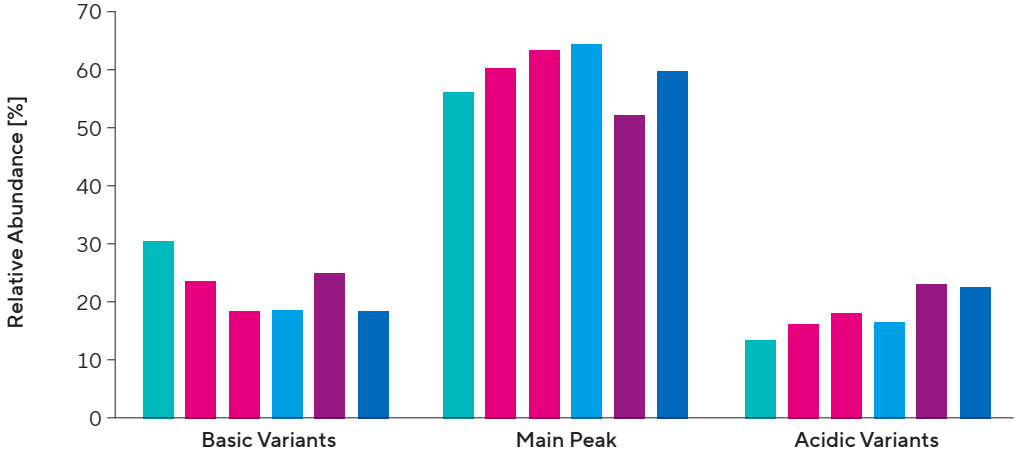
Note. One replicate is displayed as diamonds; the other replicate is displayed as squares.



- 4Cell® CHO Medium TCX6D with 4Cell® CHO Feed TCX7D
- 4Cell® CHO Medium TCX6D with 4Cell® SmartCHO FMA and FMB
- 4Cell® CHO-GS Medium TCX10D with 4Cell® SmartCHO FMA and FMB
- 4Cell® SmartCHO Production Medium with 4Cell® SmartCHO FMA and FMB
- 4Cell® CHO Medium TCX6D with 4Cell® CHO Feed TCX7D and 4Cell® SmartCHO FMB



- 4Cell® CHO Medium TCX6D with 4Cell® CHO Feed TCX7D
- 4Cell® CHO Medium TCX6D with 4Cell® SmartCHO FMA and FMB
- 4Cell® CHO-GS Medium TCX10D with 4Cell® SmartCHO FMA and FMB
- 4Cell® SmartCHO Production Medium with 4Cell® SmartCHO FMA and FMB
- 4Cell® CHO Medium TCX6D with 4Cell® CHO Feed TCX7D and 4Cell® SmartCHO FMB



- 4Cell® CHO Medium TCX6D with 4Cell® CHO Feed TCX7D
- 4Cell® CHO Medium TCX6D with 4Cell® SmartCHO FMA and FMB
- 4Cell® CHO-GS Medium TCX10D with 4Cell® SmartCHO FMA and FMB
- 4Cell® SmartCHO Production Medium with 4Cell® SmartCHO FMA and FMB
- 4Cell® CHO Medium TCX6D with 4Cell® CHO Feed TCX7D and 4Cell® SmartCHO FMB

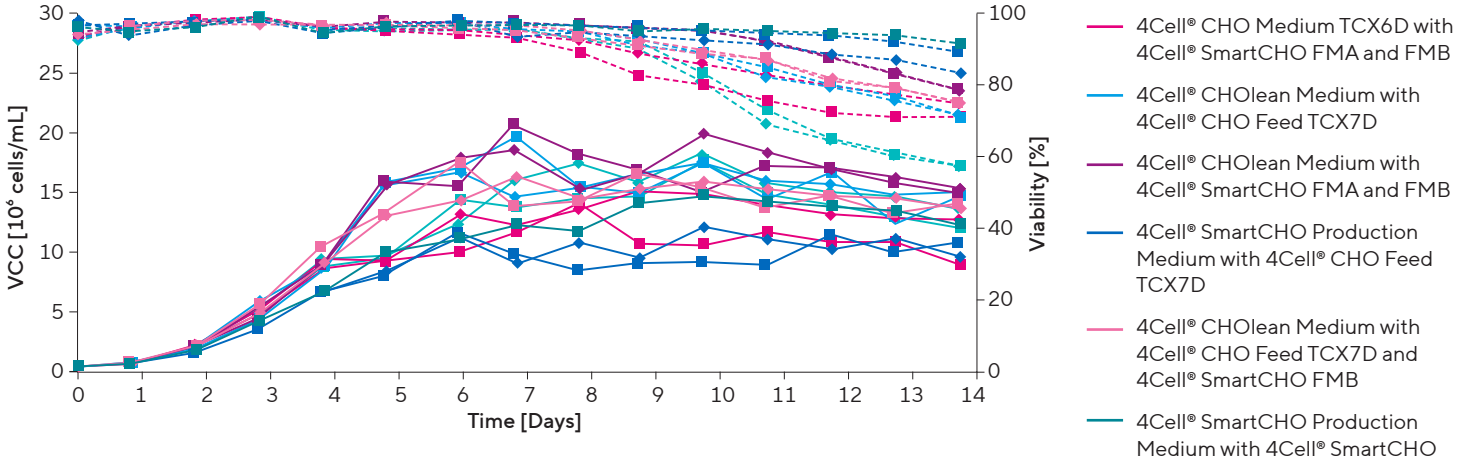
All tested media and feed combinations achieved high maximum viable cell concentrations (VCCs) exceeding 25 × 10^6 cells/mL. The 4Cell® CHO Medium TCX6D and 4Cell® SmartCHO FMA + 4Cell® SmartCHO FMB combination achieved the highest VCCs and integrated VCC (IVCC;data not shown).

Following the CHO-K1 evaluation, a media–feed screening was carried out in Ambr® 15 with an IgG-expressing CHO-S cell line, using glucose as the lead substrate to guide the feeding strategy (Figure 12). The choice of production medium had a substantial impact on titer, with 4Cell® CHOlean Medium, outperforming all other media. In addition, the 4Cell® SmartCHO feeds consistently provided higher titers compared to 4Cell® CHO Feed TCX7D across all media. Thus, the best performance was achieved with the 4Cell® CHOlean Medium with 4Cell® SmartCHO FMA + 4Cell® SmartCHO FMB combination, resulting in an average titer of 3.227 g/L.

The combination of 4Cell® CHOlean Medium with 4Cell® SmartCHO FMA and FMB feeds was scaled to a 2 L Biostat® bioreactor to characterize the process at a more clinically-relevant scale. Substantially higher cell densities were observed compared to Ambr® 15 cultivation, resulting in a 1.4-fold higher product titer of 4.5 g/L and a depletion of glucose on day 7 (data not shown).

Figure 12: *CHO-S Viable Cell Density and Viability, Antibody Titers, Glycosylation Profiles, and Charge Variants With Different Media | Feed Combinations*

A



Note. One replicate is displayed as diamonds; the other replicate is displayed as squares.

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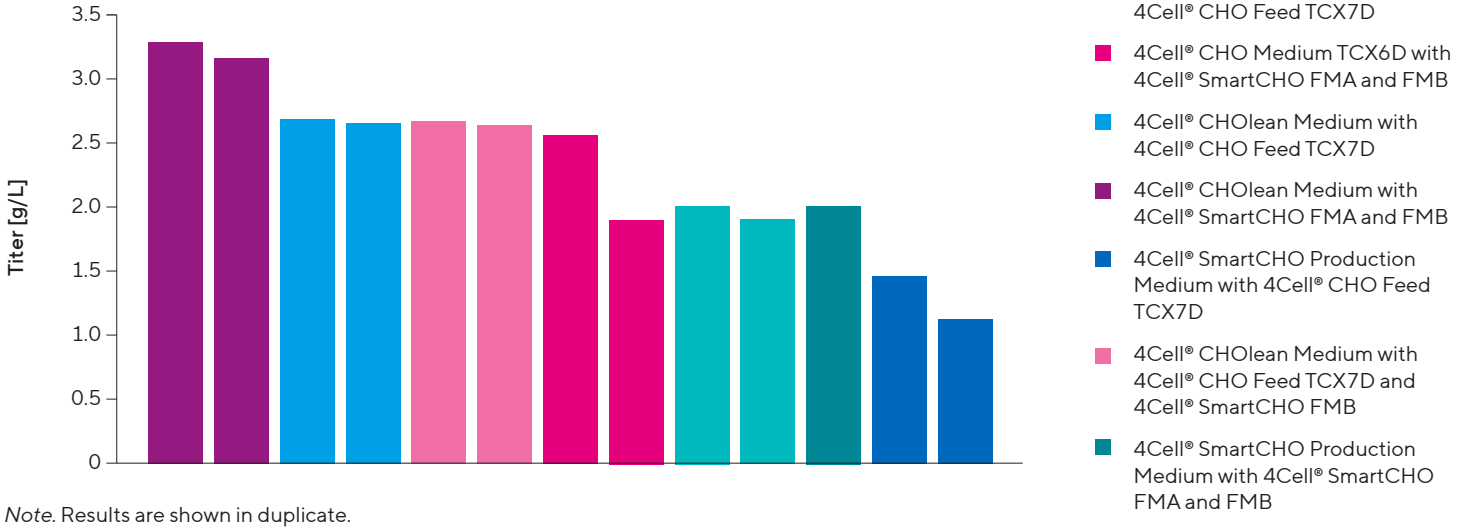
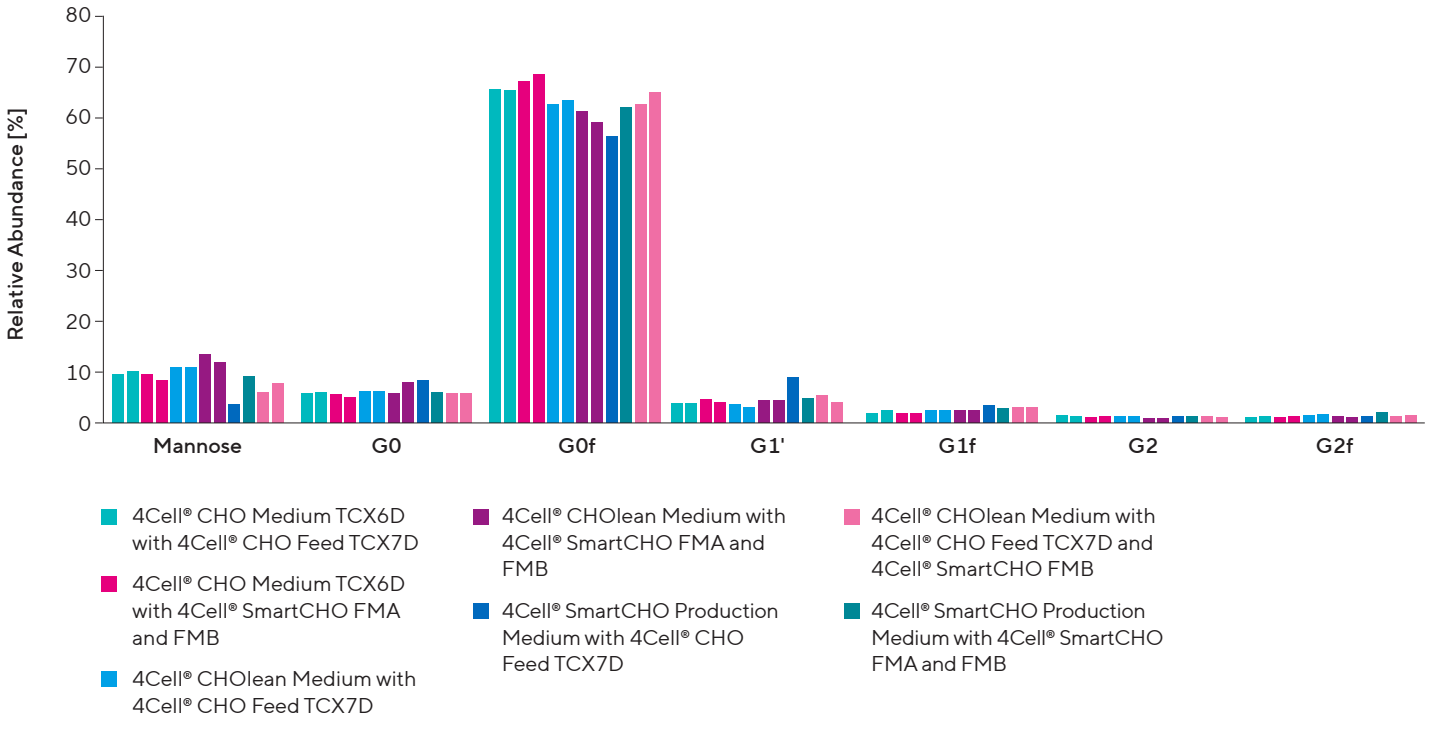


Note. Results are shown in duplicate.

C



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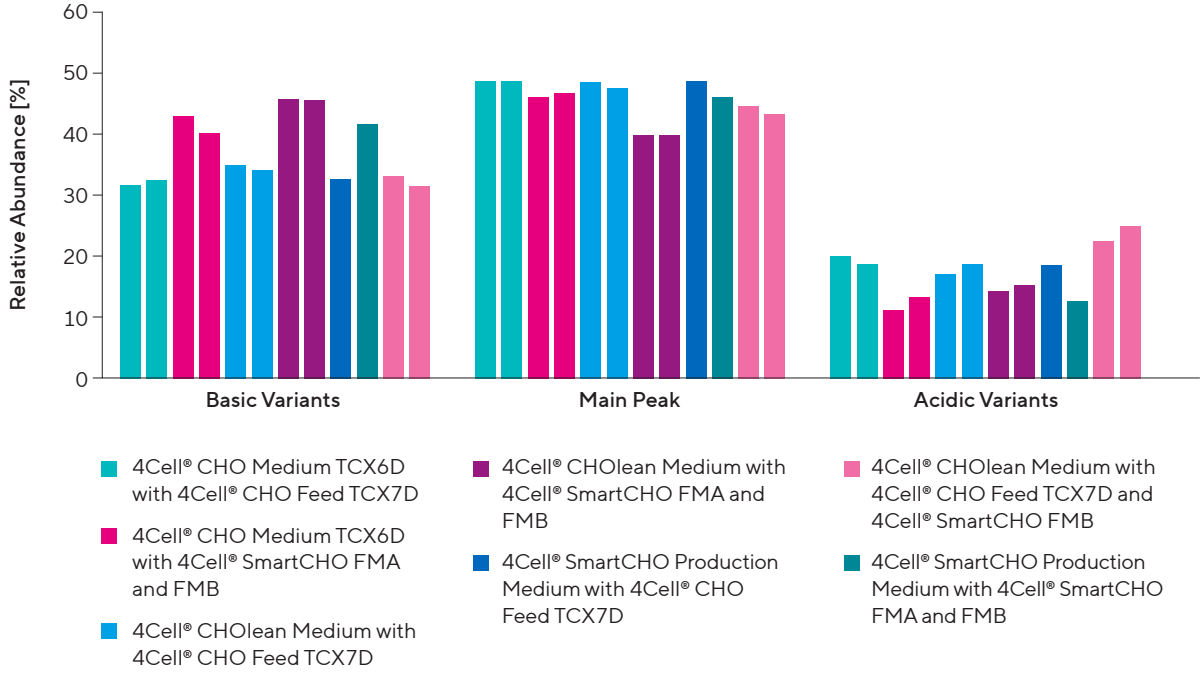


Note. Results of Ambr® 15 fed-batch media-feed screening experiment depicting growth curves of CHO-S cultures in different media | feed combinations (solid line = VCC, dashed line = viability) **(A)**, IgG titer **(B)**, product N-glycosylation profiles **(C)**, and charge variant heterogeneity **(D)** in final day samples. Experiments were conducted in duplicate (same color). No PQA analytics were performed on the 4Cell® SmartCHO Production Medium and 4Cell® CHO Feed TCX7D duplicate with the lowest titer due to capacility limitations.

4Cell® CHO Medium TCX10D — A High-Performing Medium for CHO-GS Cell Lines

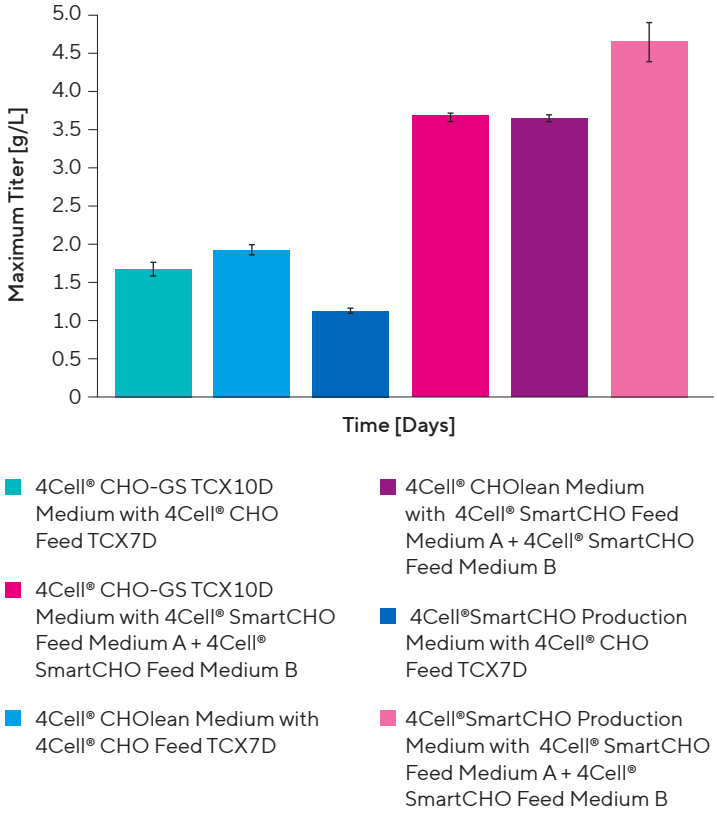
CHO-GS cells (stably producing an IgG antibody product) were cultivated in different media in an Ambr® 15 bioreactor, where each medium was combined with two different feed systems, including 4Cell® SmartCHO FMA and 4Cell® SmartCHO FMB (Figure 13). Feeds were added to the Ambr® 15 based on a trigger-target step, with glucose concentrations set according to pre-culture growth, glucose consumption, and customer process information.

All media-feed combinations supported high cell growth and densities, with 4Cell® CHO-GS Medium TCX10D and 4Cell® SmartCHO Production Medium achieving higher cell densities when combined with 4Cell® SmartCHO FMA and FMB. Antibody titers were significantly higher in cultures with the 4Cell® SmartCHO feed, exceeding 4.5 g/L, compared to a maximum of 2 g/L with 4Cell® CHO Feed TCX7D.

Transient Transfections

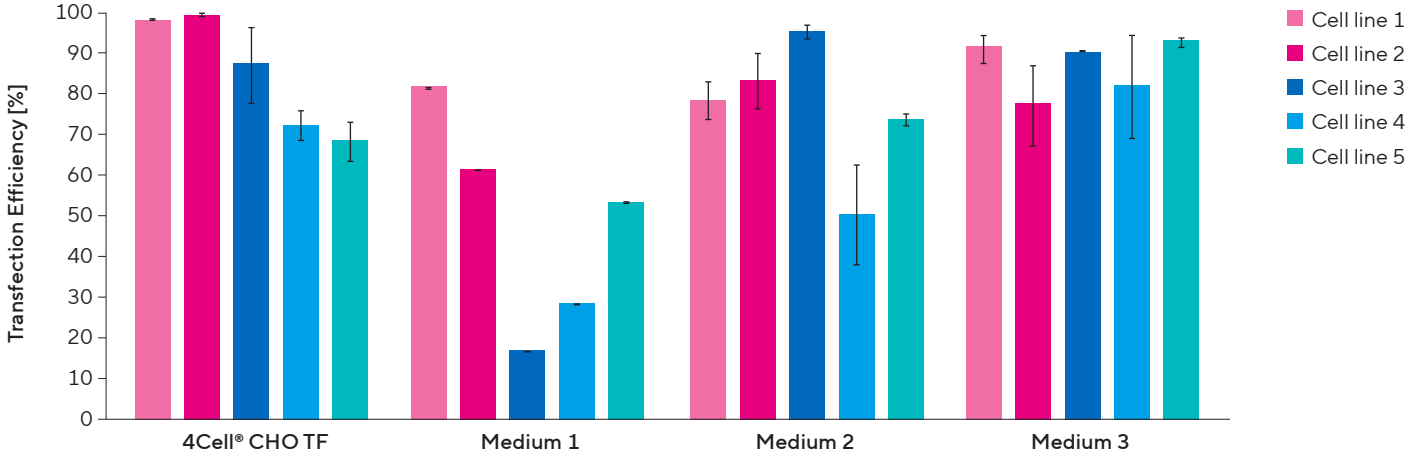
4Cell® CHO TF Medium is the designated medium in our portfolio for supporting transient expression. It is formulated without components that may interfere with the transfection complex, ensuring efficient transfection and expression (Figure 14). Note that transient expression processes are usually carried out in a batch mode expression rather than a fed-batch process mode, as it is inefficient to prolong expression over several days, and typically short timelines are required.

Figure 13: *CHO-GS Cell Antibody Titers With Different Media | Feed Combinations*



Note. Data are from duplicates.

Figure 14: *Transfection Efficiency Observed in Five CHO Cell Lines in 4Cell® CHO TF Medium vs. Commercial Media*



Note. Transfection efficiency was measured using GFP expression. Commercial media were current state-of-the-art chemically defined expression media for CHO suspension cells. Fresh medium was used at the time of transfection. Data are from duplicates.

Conclusion

This application note details experiments evaluating and optimizing the Sartorius 4Cell® CHO media portfolio for CHO cell cultivation. Using the Ambr® 15 system, various proprietary CHO media and feed solutions were tested across different CHO cell lines, including CHO-DG44, CHO-K1, CHO-S, and CHO-GS. The study focused on fed-batch, HIFB, N-1 perfusion, and continuous perfusion processes.

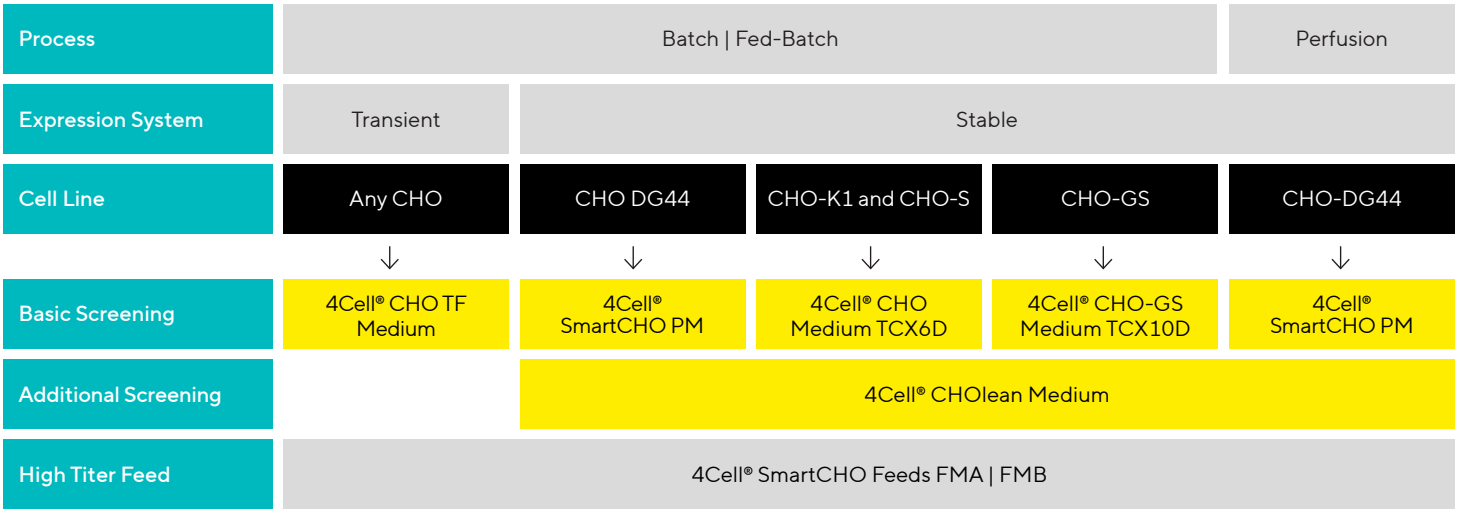
The results showed robust growth and high productivity, particularly with 4Cell® SmartCHO Production Medium for CHO-DG44 cells, 4Cell® CHO Medium TCX6D for CHO-K1 and CHO-S cells, and 4Cell® CHO-GS TCX10D Medium for CHO-GS cells, achieving high titers when combined with the 4Cell® SmartCHO feeds. The experiments demonstrated the media's efficacy in supporting high-density cultures and intensified manufacturing strategies, highlighting Sartorius media as versatile solutions for optimizing protein production processes.

The decision tree in Figure 15 provides a framework for enhancing CHO media screening success by using two pre-selected media and feed combinations. It details strategic approaches for optimizing cell culture conditions through batch, fed-batch, and perfusion methods, commonly used in stable expression systems. The 4Cell® CHO TF Medium is tailored for transient protein expression in any CHO cell line, especially effective with FectoPRO® transfection reagents.

For stable expression, 4Cell® SmartCHO Production Medium supports CHO-DG44 cell lines in fed-batch and perfusion processes. In contrast, 4Cell® CHO Medium TCX6D is designed for CHO-K1 and CHO-S cells, and 4Cell® CHO-GS TCX10D Medium supports CHO-GS cells. 4Cell® CHOlean Medium offers a lean, robust, and flexible basis with balanced components, competing with advanced media in cell growth and product titer, recommended as a secondary choice for media screening.

Finally, 4Cell® SmartCHO FMA and FMB have consistently demonstrated their effectiveness as the optimal feeds for any basal media, significantly enhancing performance and yielding higher titers.

Figure 15: *Selection of 4Cell® CHO Media and Feeds to Guide the Initial Media Selection Process*



Find more information, visit CHO Media | Sartorius or contact your Sales Representative.

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