

Optimizing Feed Strategies with Cellvento® ModiFeed Prime COMP: A Scalable Process

Development of upstream processes, that maximize productivity without increasing complexity, relies on the right combination of medium and feed and the ability to maintain tight control of culture conditions. In fed-batch mode, cells are initially grown in a relatively lean production medium followed by regular feed supplementation to achieve the desired product yield.

Cellvento® ModiFeed Prime COMP is a chemically-defined (CD), highly concentrated, single part, pH-neutral feed for replenishing depleted nutrients required for cellular function and to extend the production phase in fed-batch mode. It is formulated for fed-batch production of monoclonal antibodies (mAbs) and next-generation biopharmaceuticals in all Chinese Hamster Ovary (CHO) cell-based expression systems, in

combination with Cellvento® 4CHO COMP and EX-CELL® Advanced CHO production media, or many leading base media for CHO cells.

Use of this feed reduces workflow complexity and risk to the process. The feed is supplied as a compacted dry powder which reduces the bulk volume, improves flowability, decreases dust formation, simplifies handling and accelerates dissolution. Once hydrated, the feed can be stored protected from light at 2-8°C for up to 90 days or at room temperature for up to 30 days.

The advantages of Cellvento® ModiFeed Prime COMP are summarized in **Table 1**.

Cellvento® ModiFeed Prime COMP Feature	Advantage
All components are of non-animal origin (NAO) and the feed does not contain hydrolysates, phenol red or 2-mercaptoethanol	Minimizes risk of potential viral contamination and ensures batch-to-batch consistency
High concentration formulation (> 131 g/L)	Minimizes total feed volume (15-27.5%), resulting in maximized bioreactor working volume, decreased product dilution, and increased yield
Exclusion of glucose from the formulation	Allows full control of the glucose feeding strategy
Single part feed containing modified amino acids and amino acid derivatives	Reduces the complexity of hydration, storage, and feeding and eliminates the need for a second high pH feed
Doesn't require pH adjustment and has a neutral final pH	Eliminates use of acids or bases in hydration and the influence of high or low pH feeds on the fed-batch process
Supplied as a compacted dry powder	Convenient to use
Hydrated feed can be stored protected from light at 2-8 °C for up to 90 days or at room temperature for up to 30 days	Increases storage and use options and reduces contamination risk by limiting bioreactor connections when stored at room temperature

Table 1. Cellvento® ModiFeed Prime COMP offers significant advantages for fed-batch processes.

Performance Highlights

Cellvento® ModiFeed Prime COMP supports optimal cell growth and high productivity of a range of suspension CHO cell lines such as CHO-K1, CHO-DG44, CHO-S and CHO-GS, including cell lines derived using the CHOZN® platform. As shown in **Figure 1**, this feed supports cell growth and productivity in different base media; the specific base media, however, may impact performance metrics.

Two cell lines expressing mAbs, CHOZN® mAb1 and mAb2, were cultured in spin tubes containing either EX-CELL® Advanced CHO or Cellvento® 4CHO COMP fed-batch medium.

Cellvento® ModiFeed Prime COMP was fed at 17.5% total volume. Similar peak viable cell density (VCD) was observed for CHOZN® mAb1 in both media while the viability was better maintained, and productivity was highest in Cellvento® 4CHO COMP. CHOZN® mAb2 experienced higher peak VCD in EX-CELL® Advanced CHO fed-batch medium and this increase in VCD correlated with increased production. While both EX-CELL® Advanced CHO and Cellvento® 4CHO COMP fed-batch media performed well, a clonal preference was clearly demonstrated (**Figure 1B**).

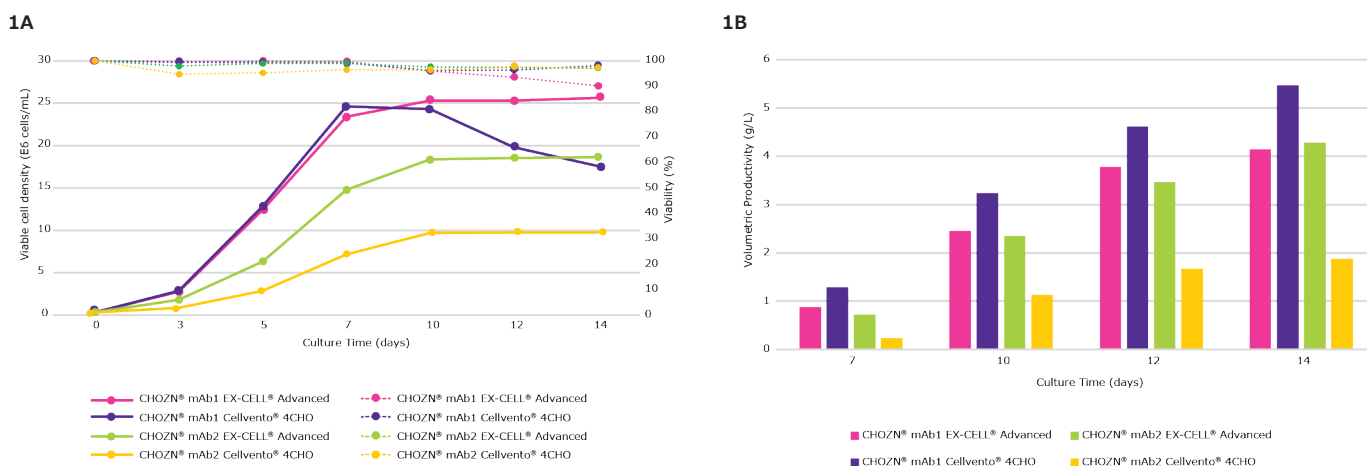


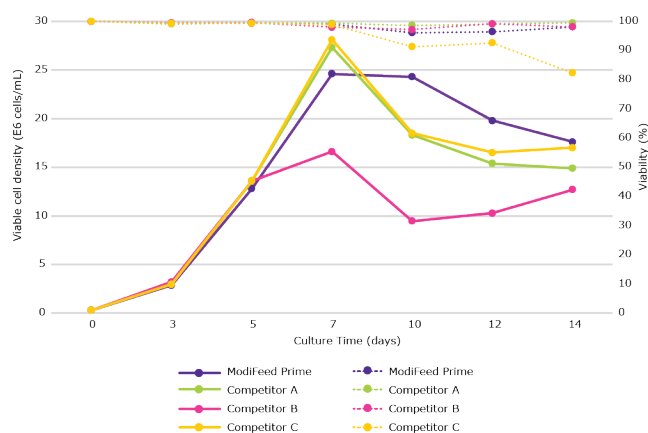
Figure 1. Cellvento® ModiFeed Prime COMP performance with different base media. Viable cell density (1A solid lines), viability (1A dashed lines), and volumetric productivity (1B) during a 14 day fed-batch process for CHOZN® mAb1 and mAb2 cultured in EX-CELL® Advanced CHO fed-batch medium and Cellvento® 4CHO COMP.

Cellvento® 4CHO COMP was shown to be the best medium for use in conjunction with CHOZN® mAb1 (**Figure 1B**). Cellvento® ModiFeed Prime COMP represents the optimum feed for use in combination with Cellvento® 4CHO COMP. **Figure 2** compares Cellvento® ModiFeed Prime COMP and other commercially available feeds in spin tube cultures of CHOZN® mAb1 using Cellvento® 4CHO COMP.

Cellvento® ModiFeed Prime COMP was fed at 17.5% total feed, while other feeds were fed according to

the primary recommendations in their respective product literature. While Cellvento® ModiFeed Prime COMP did not produce the highest peak viable cell density, it maintained a high viable cell density for a longer period than Competitors A and C (**Figure 2A**). Cellvento® ModiFeed Prime COMP yielded the highest volumetric productivity on all days analyzed. (**Figure 2B**). Competitor B produced the lowest peak viable cell density and subsequently the lowest volumetric productivity.

2A



2B

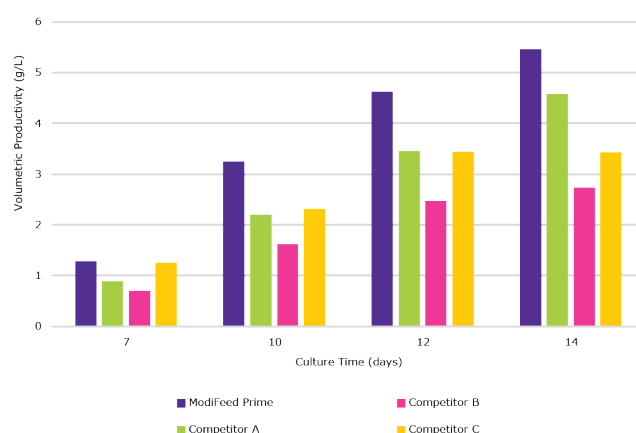


Figure 2. Cellvento® ModiFeed Prime COMP performance compared to other commercially available feeds. Viable cell density (2A solid lines), viability (2A dashed lines), and volumetric productivity (2B) during a 14 day fed-batch process for CHOZN® mAb1 cultured in Cellvento® 4CHO COMP supplemented with either Cellvento® ModiFeed Prime COMP or other commercially available feeds.

Cellvento® ModiFeed Prime COMP Feeding Strategy

As with most upstream bioprocesses, optimization of feed volumes and timing of feed administration should be empirically determined on a process- and cell-line specific basis to maximize performance. Cellvento® ModiFeed Prime COMP, used in conjunction with Cellvento® 4CHO COMP or EX-CELL® Advanced CHO fed-batch medium, is recommended to be fed at between 15% and 27.5% total feed, depending on the demands of the clone(s) tested. **Table 2** provides the recommended ranges for evaluation of feed percentage, feed frequency and glucose concentration.

Feeding should be initiated only when the viable cell density is $\geq 2 \times 10^6$ cells/ml and no earlier than day 3 to avoid over-feeding. Cultures should be terminated and harvested when viability drops below 70%.

Initial evaluations should consist of 17.5% and 22.5% total feed unless the general demands of the clone(s) are known (**Table 3A**). Once a preference for a lower or higher total feed percentage is established optimization through further increasing or decreasing the total feed can be evaluated. If desired, the schedule can be adjusted to support a more regular feed interval (**Table 3B**).

Parameter	Recommended Range for Evaluation
Cellvento® ModiFeed Prime COMP	2.5% – 7.5% (v/v)
Frequency	48 - 72 hour feed intervals
Glucose	4 – 6 g/L monitor daily

Table 2. Guidelines for optimizing the feeding strategy.

3A

Culture Day	Total Feed	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total Feed
Cellvento® ModiFeed Prime COMP (% v/v)	Low				3		3		5.5			3		3			17.5
Cellvento® ModiFeed Prime COMP (% v/v)	High				4		4		6.5			4		4			22.5

3B

Culture Day	Total Feed	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total Feed
Cellvento® ModiFeed Prime COMP (% v/v)	Low				3.5		3.5		3.5		3.5		3.5				17.5
Cellvento® ModiFeed Prime COMP (% v/v)	High				4.5		4.5		4.5		4.5		4.5				22.5

Table 3. Low and high ranges for optimizing the feed strategy (3A) and an option for a more regular feed interval (3B).

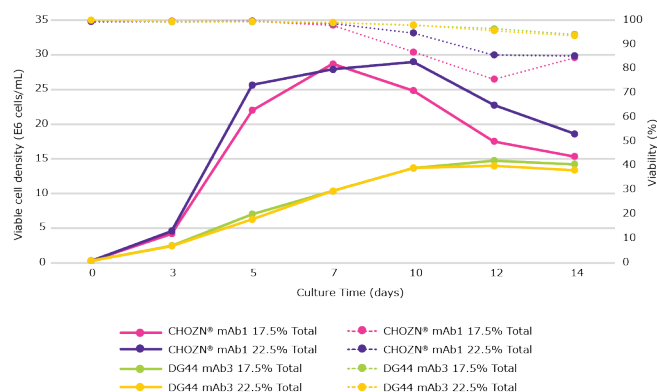
The suggested initial feeding evaluation is designed to support a low seed ($2-5 \times 10^5$ cells/mL), 14 day fed-batch process. If viability is still high late in culture, longer fed-batch processes can be supported with additional feeding; the feeding frequency and percentage is then continued until viability drops below 80%. A high seed fed-batch ($2-5 \times 10^6$ cells/mL) may require adjustment in feeding schedule to support higher biomass earlier in culture and provide more total feed. Feeding can be initiated as early as day 2 to support higher biomass early in culture. High seed may represent an option for shortening the duration of longer fed-batches or for achieving increased production over the same 14 days.

Testing multiple feed strategies is important as increased feeding may result in productivity gains or may have a neutral or even negative effect on productivity for less demanding clones (**Figure 3**). CHOZN[®] mAb1 and DG44 mAb3 were cultured in

spin tubes using Cellvento[®] 4CHO COMP and EX-CELL[®] Advanced CHO fed-batch media, respectively. Cellvento[®] ModiFeed Prime COMP was fed at 17.5% total feed (3% on days 3 and 5, 5.5% on day 7, and 3% on days 10 and 12) or 22.5% total feed (4% on days 3 and 5, 6.5% on day 7, and 4% on days 10 and 12). Cellvento[®] ModiFeed Prime COMP enables all high-performing CHO cell lines, CHOZN[®] and DG44 shown here, to achieve their full production potential.

Similar peak VCDs were observed for both clones, no matter the feeding strategy (**Figure 3A**). CHOZN[®] mAb1 maintained a higher viability and VCD when fed at 22.5% total feed which led to a higher peak volumetric productivity. The amount of total feed had no impact on the VCD and viability of DG44 mAb3. An increase in total feed had a significantly higher impact on the productivity of CHOZN[®] mAb1 compared to DG44 mAb3 (**Figure 3B**).

3A



3B

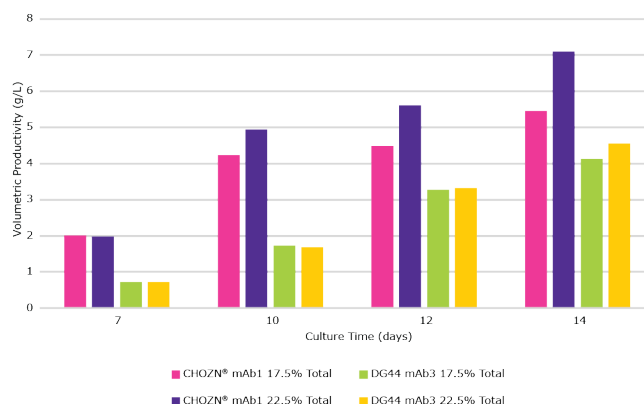


Figure 3. The impact of feeding schedules with Cellvento[®] ModiFeed Prime COMP using different cell lines. Viable cell density (3A solid lines), viability (3A dashed lines), and volumetric productivity (3B) during a 14 day fed-batch process for CHOZN[®] mAb1 cultured in Cellvento[®] 4CHO COMP and DG44 mAb3 cultured in EX-CELL[®] Advanced CHO fed-batch medium.

Scalability

Use of the Cellvento[®] ModiFeed Prime COMP is scalable, delivering comparable results in terms of growth, viability, productivity, glycosylation and charge variance (**Figure 4**). CHOZN[®] mAb1 was cultured in spin tubes, 3L Mobius[®] single-use bioreactors and a 50L Mobius[®] single-use bioreactor in EX-CELL[®] Advanced CHO Fed-Batch Medium (**Table 4**). Cellvento[®] ModiFeed Prime COMP was fed at 25% total feed (4.5% days 3 and 5, 7% day 7, 4.5% days 10 and 12). A high total feed percentage was defined as optimal for this clone (**Figure 3B**) and further optimized as noted under feeding strategy (data not shown). EX-CELL[®] Advanced CHO Fed-Batch Medium was used in order to confirm

that an increase in productivity, over the 17.5% total feed (**Figure 1B**), would be observed in both media when using a higher total feed percentage. CHOZN[®] mAb1 cultured in a 50L Mobius[®] single use bioreactor saw slight increases in peak viable cell density, longevity, and productivity over the 3L Mobius[®] single use bioreactors and spin tubes.

Only subtle changes in the glycoprofiles (**Figure 4C**) were observed across the three scales tested. The use of single use Mobius[®] bioreactors, as opposed to spin tubes, generated an increase in acidic and a decrease in main charge variants (**Figure 4D**).

Experimental Condition	Operating Parameter	Operating Parameter	Operating Parameter
Culture Vessel	Spin Tube	Mobius® 3L Single-Use Bioreactor	Mobius® 50L Single-Use Bioreactor
Initial Working Volume	30 mL	1.4 L	32.6 L
Inoculation Density	5 x 10 ⁵ viable cells/mL	5 x 10 ⁵ viable cells/mL	5 x 10 ⁵ viable cells/mL
Temperature	37°C	36.8°C	36.8°C
Agitation	320 rpm (25 mm orbit)	~ 14 W/m ³ (200 rpm)	~ 14 W/m ³ (150 rpm)
DO setpoint	NA	50%	50%
Sparging strategy	NA	O ₂ through open pipe	O ₂ through open pipe
pH set point/range	NA	6.9 (+ 0.10 / - 0.15)	6.9 (+ 0.10 / - 0.15)
pH control	5% CO ₂	CO ₂ and Na ₂ CO ₃	CO ₂ and Na ₂ CO ₃
Sampling Points	Study Days 0,3,5,7,10,11,12,14	Study Days 0,3,4,5,6,7,10,11,12,13,14	Study days 0,3,4,5,6,7,10,12,13,14
Sampling Volume	0.5-2 mL per day (depending on analysis needed)	6-25 mL per day (depending on analysis needed)	10-25 mL per day (depending on analysis needed)
Glucose Addition	On sample days, feeding to levels ≥ 6 g/L	On sample days, feeding to levels ≥ 6 g/L	On sample days, feeding to levels ≥ 6 g/L

Table 4. Experimental conditions and operating parameters: spin tubes, 3L and 50L bioreactors.

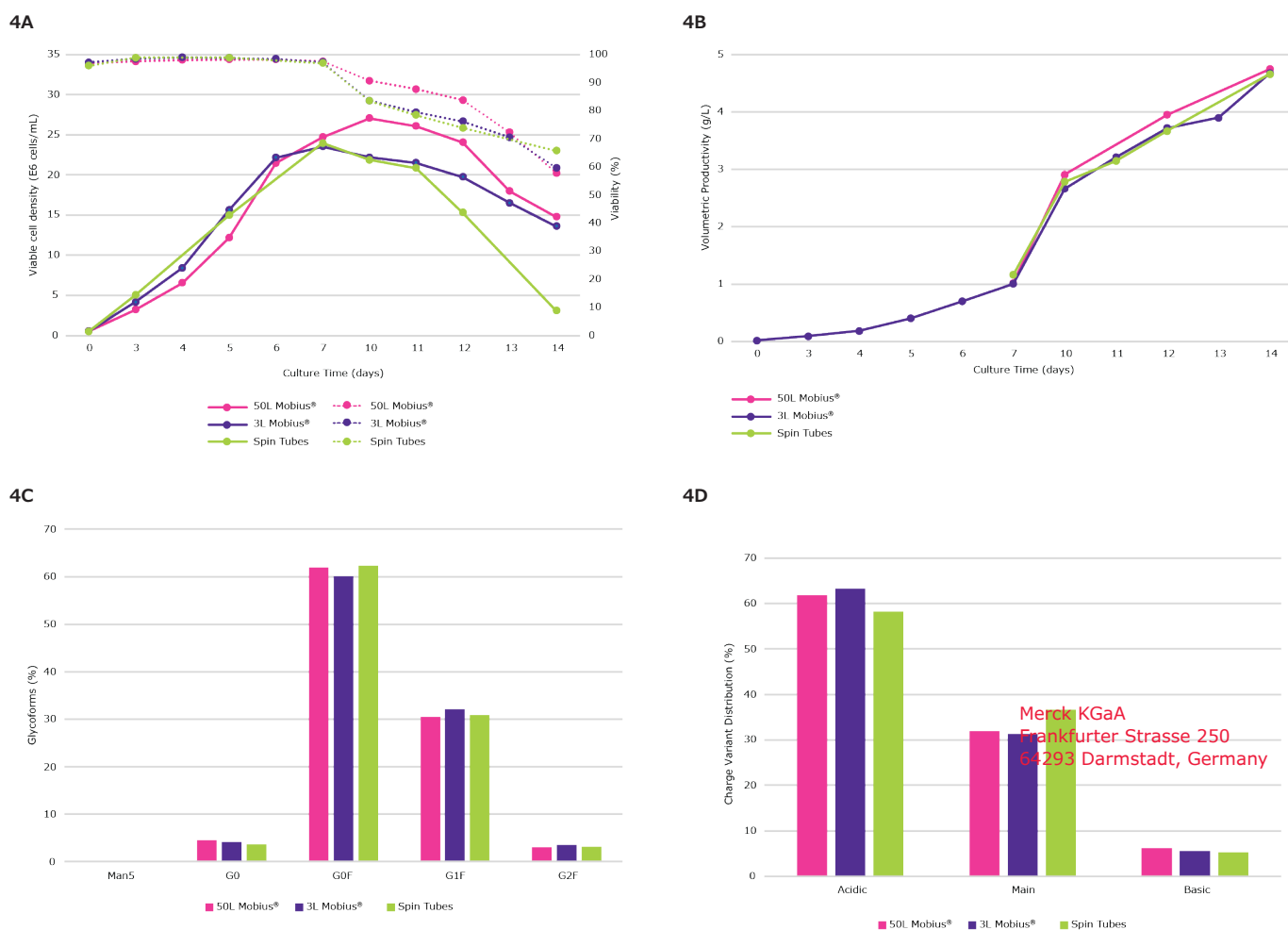


Figure 4. ModiFeed Prime COMP performance at different scales: spin tubes, 3L and 50L bioreactors. Viable cell density (4A solid lines), viability (4A dashed lines), volumetric productivity (4B), day 14 glycoprofile (4C) and day 14 charge variant distribution (4D) during a 14 day fed-batch process for CHOZN® mAb1 cultured in EX-CELL® Advanced CHO fed-batch medium. Cultures were grown in spin tubes, 3L Mobius® single-use bioreactors and a 50L Mobius® single-use bioreactor.

Conclusion

Development of upstream processes requires careful orchestration of medium and feed formulations to optimize culture conditions. Cellvento® ModiFeed Prime COMP can play an integral role in the development of upstream processes that maximize productivity without resulting in increased complexity. This feed is compatible with a range of suspension CHO cell lines and base medium, and when used in combination with Cellvento® 4CHO COMP and EX-CELL® Advanced CHO media, boosts productivity beyond that of current products.

In addition to robust performance, the format in which the feed is supplied allows for easier handling and a more streamlined workflow. The product is provided in a compacted format as a single part, pH-neutral feed; there is no need to control pH effects caused by non-neutral pH feeding. Once hydrated, the feed is stable protected from light for up to 90 days at 2-8°C or up to 30 days at room temperature, providing increased flexibility in terms of storage and minimizing contamination risks as a result of reduced handling requirements.

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