

Bioproduction

Consistent improvement in CHO-K1 GS performance with Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer

Keywords

CHO-K1 GS, CHO GS, CHO feed, CHO medium, cell culture, fed-batch

Introduction

Technological advancements have provided a better understanding of the nuanced performance requirements across various bioproduction CHO cell lines. The knowledge gained has helped enable the development of an improved platform medium and feeds to better accommodate the specific needs of various CHO cell lines. The Gibco™ Efficient-Pro™ system was designed to provide a platform medium, Efficient-Pro™ Medium, and a family of feeds formulated to improve the bioproduction performance of specific CHO cell lines. Improved cell growth performance has been shown when Efficient-Pro Medium is paired with Gibco™ Efficient-Pro™ Feed 1 for CHO-K1 cells and some CHO-K1 GS cell lines, or when the medium is paired with Gibco™ Efficient-Pro™ Feed 2 for CHO-S and DG44 cells and some CHO-K1 GS cell lines.

Bioproduction manufacturers are moving to more advanced cell lines to further optimize protein productivity. However, the more advanced cell lines, specifically CHO-K1 GS cells, present unique nutritional and metabolic requirements that must be met to improve productivity [1]. Using advanced media development technologies, including multi-omics and high-throughput screening, a two-part feed system was developed to support this cell line's requirements and help deliver consistently improved performance. As part of the Gibco Efficient-Pro family, Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer are

designed for optimal CHO-K1 GS performance when paired with Efficient-Pro Medium. They are chemically defined (CD) and animal origin-free (AOF) formulations that are offered in multiple product formats and packaging options to support various workflows and scalability at each bioproduction stage.

To evaluate the performance of Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer, fed-batch 14-day studies were conducted with IgG-producing CHO-K1 GS cells (HealSun) in Ambr™ 15 (15 mL) microbioreactors (Sartorius) and 3 L Thermo Scientific™ HyPerforma™ Glass Bioreactors, as outlined in Table 1. To assess the consistency of performance across scales, Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer was evaluated with multiple runs in the Ambr 15 microbioreactor study and compared to multiple runs in the 3 L study with assessment of viable cell density (VCD), cell viability, and IgG titer. In addition, the 3 L study evaluated the use of Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer relative to Efficient-Pro Feed 2, which was previously determined to perform best for this specific CHO-K1 GS cell line. Feed performance was assessed by comparing cell growth, productivity, and key product quality attributes, including aggregation and N-glycans.

Table 1. Materials and methods used to evaluate performance of media and supplements.

Parameter	Ambr 15 microbioreactor study	3 L bioreactor study
Runs and replicates	3 runs with triplicate vessels per run	3 runs with duplicate vessels per run
Medium and supplementation	Efficient-Pro Medium with 1X final concentration of GS Supplement 50X (Millipore Sigma) and 1% Gibco™ Anti-Clumping Agent	
Feeds and feeding strategies	2.6% Efficient-Pro Feed 3 with 0.26% Efficient-Pro Feed Enhancer, fed daily on days 3–13	2.6% Efficient-Pro Feed 3 with 0.26% Efficient-Pro Feed Enhancer, fed daily on days 3–13 or 2% Efficient-Pro Feed 2, fed daily on days 3–13
	Glucose maintained at ≥3 g/L	Glucose maintained at ≥2 g/L
Seeding density	0.3 x 10 ⁶ viable cells/mL	
VCD and cell viability	Vi-CELL™ XR Cell Viability Analyzer (Beckman Coulter)	
IgG titer	Cedex™ Bio HT Analyzer (Roche)	
Aggregation	--	Size exclusion high-performance liquid chromatography (HPLC)
N-glycans	--	Reductive amination labeling and hydrophobic interaction liquid chromatography (HILIC) ultrahigh-pressure liquid chromatography (UPLC)

Results

Across multiple runs, Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer supported consistently high cell growth, with average VCDs of approximately 25–35 x 10⁶ cells/mL at the Ambr 15 microbioreactor scale and approximately 40–45 x 10⁶ cells/mL at the 3 L bioreactor scale (Figure 1). For productivity, Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer demonstrated consistently high IgG titers at both scales, with an average of 4.8 g/L in the Ambr 15 cultures and 5.1 g/L in the 3 L bioreactors (Figure 2).

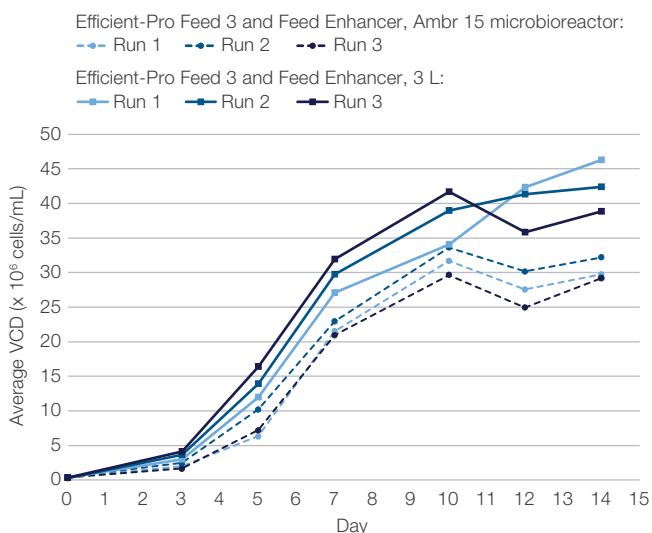


Figure 1. Cell growth across scales. Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer supported reliable high CHO-K1 GS cell growth across the two scales, with average VCDs reaching 25–35 x 10⁶ cells at the Ambr 15 microbioreactor scale and near 40–45 x 10⁶ cells/mL at the 3 L bioreactor scale. (n = 3 vessels per Ambr 15 run, n = 2 vessels per 3 L run)

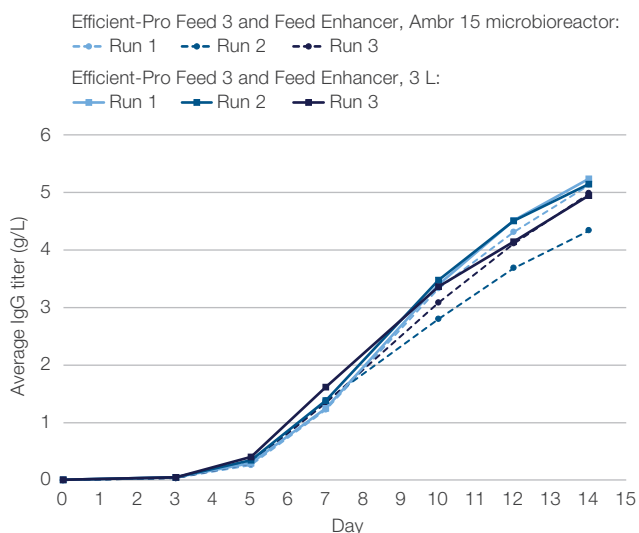


Figure 2. IgG productivity across scales. Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer enabled CHO-K1 GS cells to consistently produce close to 5 g/L average IgG titers across multiple runs, with averages of 4.8 g/L at the Ambr 15 microbioreactor scale and 5.1 g/L at the 3 L bioreactor scale. (n = 3 vessels per Ambr 15 run, n = 2 vessels per 3 L run)

In the 3 L bioreactor study, the CHO-K1 GS cell line demonstrated more consistent, although lower, average VCD across multiple runs using Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer, compared to using Efficient-Pro Feed 2 (Figure 3). Evaluation of productivity between the feed conditions showed Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer delivered a 36% higher average IgG titer of 5.1 g/L, compared to Efficient-Pro Feed 2 with an average titer of 3.8 g/L (Figure 4). Overall, product quality profiles were maintained when using Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer, compared to Efficient-Pro Feed 2 (Figure 5). In terms of aggregation, both feed systems supported the generation of similarly high levels of monomer (90% and 93% main peaks) with minimal-level ($\leq 5\%$) low molecular weight (LMW) or high molecular weight (HMW) peaks. N-glycan results showed that when using either Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer or Efficient-Pro Feed 2, similar levels of key glycan structures were maintained, including keeping Man5 glycans low at 2%. This is important, as high levels of Man5 glycans may impact monoclonal antibody (mAb) clearance and antibody-dependent cell-mediated cytotoxicity (ADCC) [2].

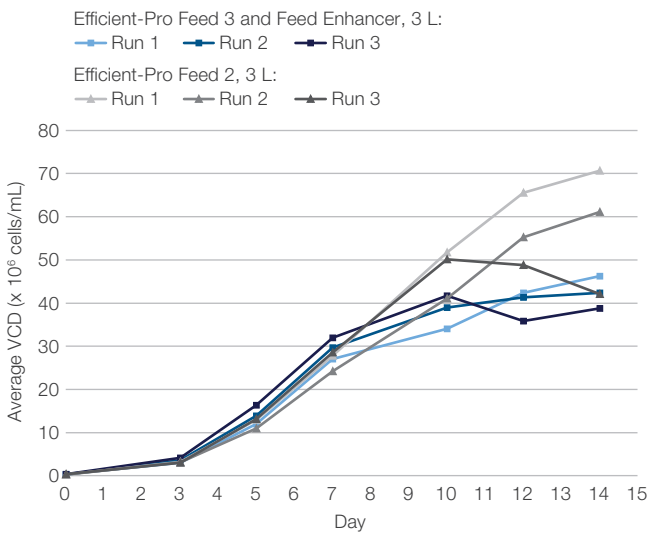


Figure 3. Cell growth between feed conditions. CHO-K1 GS cells using Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer showed more consistent VCD across multiple runs at the 3 L bioreactor scale, compared to when using Efficient-Pro Feed 2. (n = 2 vessels per 3 L run)

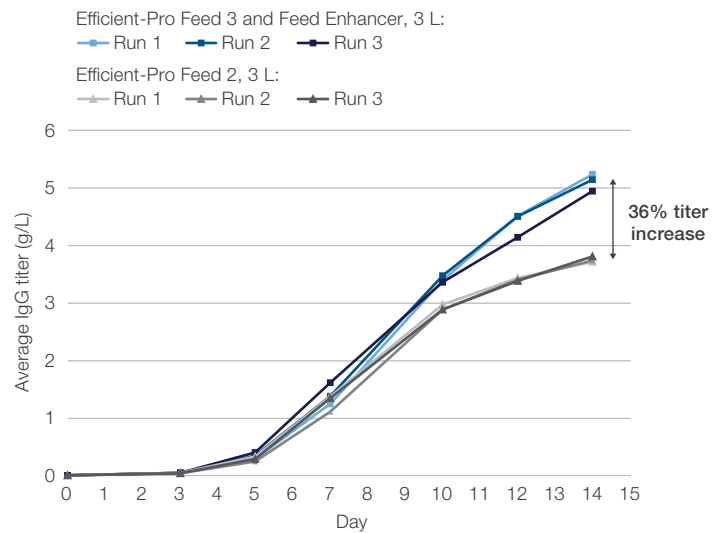


Figure 4. IgG productivity between feed conditions. An approximately 36% higher average CHO-K1 GS IgG titer of 5.1 g/L was supported using Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer across multiple runs at the 3 L bioreactor scale, relative to an average IgG titer of 3.8 g/L produced when using Efficient-Pro Feed 2. (n = 2 vessels per 3 L run)

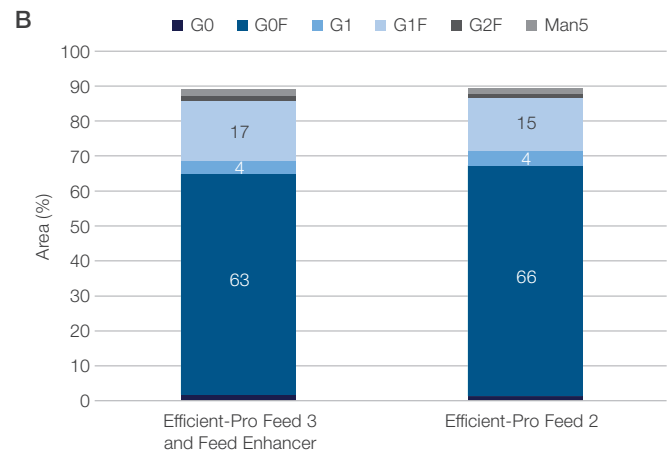
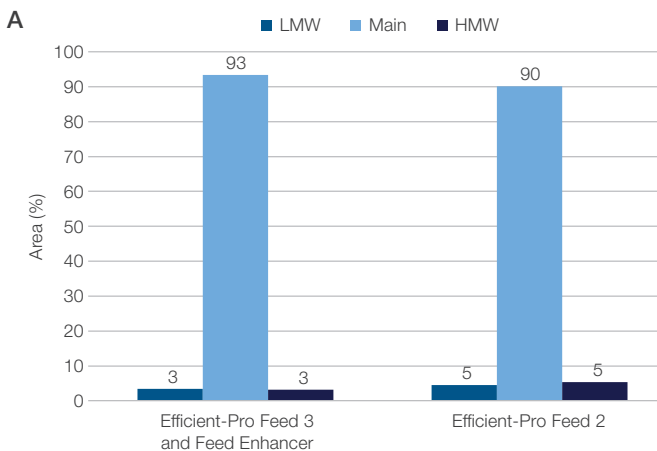


Figure 5. Product quality across feeds. Using Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer, and Efficient-Pro Feed 2 alone, shows (A) aggregation results with similarly high (90–93%) main peaks with minimal ($\leq 5\%$) low molecular weight (LMW) and high molecular weight (HMW) peaks, and (B) similar N-glycan profiles with low (2%) Man5 species.

Conclusions

The results of this study support the following benefits of using Efficient-Pro Feed 3 with Efficient-Pro Feed Enhancer for CHO-K1 GS cells:

- Consistent high cell growth and IgG productivity with scaling
- More consistent cell growth and 36% improved titer with Efficient-Pro Feed 3 with Feed Enhancer, compared to using Efficient-Pro Feed 2
- Similar minimal aggregation and N-glycan profile product quality with Efficient-Pro Feed 3 plus Feed Enhancer, compared to using Efficient-Pro Feed 2

In addition, the Efficient-Pro Medium and feed system for CHO-K1 GS cells provides CD and AOF formulations that are offered in multiple formats and packaging sizes to support bioproduction needs. Thermo Fisher Scientific provides a global network of validated equivalent manufacturing facilities and robust quality systems that help support supply assurance and confidence in the delivery of consistent, high-quality products. Experienced field application scientists and R&D team members are available to provide consultation and guidance on product and process questions when needed.

References

1. Yang W, Zhang J, Xiao Y, Li W, Wang T (2022) Screening strategies for high-yield Chinese hamster ovary cell clones. *Frontiers in Bioengineering and Biotechnology*, 10, 858478.
2. Mastrangeli R, Palinsky W, Bierau H (2019) Glycoengineered antibodies: towards the next-generation of immunotherapeutics. *Glycobiology*, 29(3), 199–210.

Ordering information

Description	Format	Packaging size	Cat. No.
Efficient-Pro Feed 3	Liquid	1,000 mL bottle	A4000135701
	Advanced Granulation Technology™ (AGT™)	1 L equivalent container	A4000222001
		10 L equivalent container	A4000222002
		100 L equivalent container	A4000222003
Efficient-Pro Feed Enhancer	Liquid	100 mL bottle	A4000135801
	Dry powder	1 L equivalent container	A4000175501
		10 L equivalent container	A4000175502
Efficient-Pro Medium	Liquid	1,000 mL bottle	A5322201
		10 L bioprocess container	A5322202
		20 L bioprocess container	A5322203
	Advanced Granulation Technology™ (AGT™)	1 L equivalent container	A5322301
		10 L equivalent container	A5322302
		100 L equivalent container	A5322303
		500 L equivalent container	A5322304

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