Stable high-producing mAb clones in 14 weeks: the CHOvantage GS Cell Line Development Kit

The pharmaceutical industry is challenged to develop molecules rapidly and provide a cost-effective, reliable supply of monoclonal antibodies (mAbs) for growing global demand. Accelerating stable cell line development (CLD) is essential to reducing the timeline for an investigational new drug (IND) while maintaining high productivity, product quality, and regulatory compliance for mAbs [1].

Traditional workflows that rely on transient expression prior to stable cell line generation introduce variability and often fail to predict final stable clone performance. This can increase redevelopment risk and extend timelines. However, many conventional stable expression systems depend on random genomic integration that results in low frequencies of high-producing stable clones. As a result, the screening of hundreds to thousands of clones is required to identify high producers [2-4].

Transposon-based vector systems improve integration efficiency and bias insertion toward transcriptionally active genomic regions, increasing the frequency of high-producing clones and reducing screening requirements and overall development costs.

The Gibco™ CHOvantage™ GS Cell Line Development (CLD) Kit integrates a cGMP-banked CHO-K1 GS host cell line, transposon-based vector system, and optimized media and feed platform to enable rapid establishment of high-performing stable clones for scalable, regulatory-aligned development.

Results demonstrate the kit's ability to support the development of stable lead clones and creation of research cell banks (RCBs) within 14 weeks (Figure 1). Lead clone confirmation was established with scale-up testing to 3 L and 5 L bioreactors. Lastly, stability testing of multiple clones from 60 to 100 generations was completed within ~8–12 weeks.

CHOvantage GS CLD Kit key benefits

1. Accelerated stable cell line development within 14 weeks
2. Reduced screening and development costs
3. Improved productivity with >7 g/L mAb titers
4. Stability up to 100 generations
5. Process support with a comprehensive user guide and regulatory support documentation
6. Simplified, flexible licensing with royalty-free milestone-free commercial licensing options

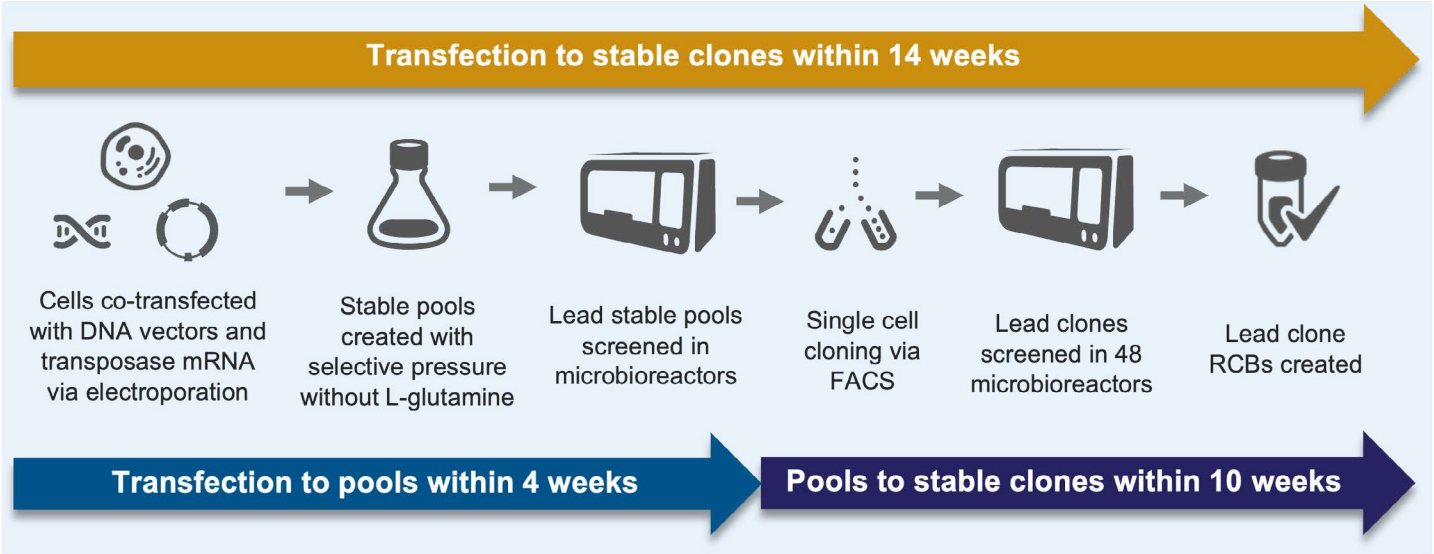

Figure 1. The CHOvantage GS CLD Kit workflow. The six-phase kit workflow, as outlined from transfection to stable lead clone RCBs, can be completed within 14 weeks. Stable pool screening and clone screening were conducted with 14-day fed-batch assays. FACS: fluorescence-activated cell sorting

Development of a stable mAb cell line

The transposon-based CHOvantage GS CLD Kit was used in a typical workflow to generate a stable mAb cell line and create RCBs within 14 weeks, as outlined in Figure 1. Scale-up testing of lead clones was performed in 3 L and 5 L bioreactors to establish and confirm the top-performing lead clone. Performance metrics, including viable cell density (VCD), cell viability, and mAb titer, were assessed at each stage. N-glycan profiles, charge variants, and aggregation were evaluated at the lead clone screening and scale-up testing. Additionally, lead clone production stability testing to a 100 population doubling level (PDL) and a 60 PDL for other screened clones was conducted within ~8–12 weeks.

Stable pool development

Stable pools were generated by co-transfecting CHOvantage GS cells with pCHOvantage DNA vectors and transposase CHOvantage mRNA, followed by applying selective pressure for ~25 days by removing L-glutamine supplementation. The lead stable pools were identified by screening for titer performance with 14-day fed-batch assays conducted in microbioreactors by three individual operators. Further assay details are in Table 1 (Materials and methods). Stable pools were generated within 4 weeks, with steady average titer results of 4.5 g/L across three operators (data not shown).

Lead clone screening

After single-cell cloning, the lead-performing clones were identified by screening for growth, productivity, and product quality using 14-day fed-batch assays in microbioreactors. Further assay details are in Table 1 (Materials and methods). Overall, strong cell growth was observed for the majority of the clones (Figure 2), with ≥5 g/L titers achieved by all lead clones (Figure 3). The two top-performing clones, A and B, achieved ≥7 g/L titers and were selected for confirmatory 3 L scale-up testing. Initial assessments of N-glycans and charge variants were considered acceptably similar to the reference standard, as shown across scales in Figure 8.

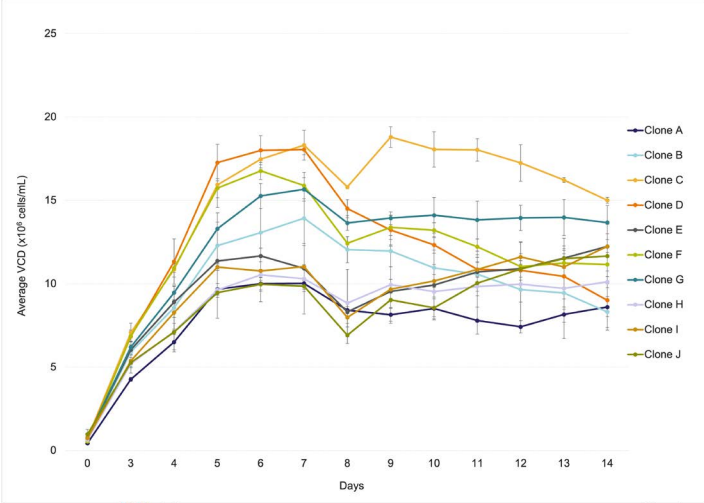

Figure 2. Lead clone screening: VCD. Strong cell growth with peak VCDs of >10 x 10^6 cells/mL was observed for seven of the ten lead clones (A–J), with all clones demonstrating high average cell viability of ≥80% through day 14 (viability data not shown). n = 3–6 microbioreactor vessels per condition

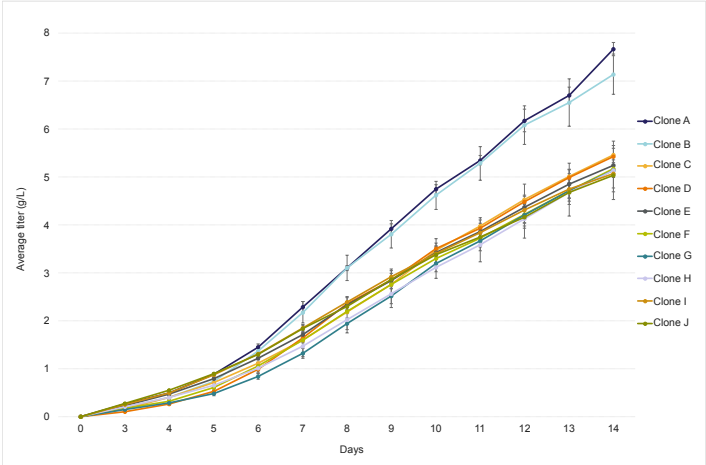

Figure 3. Lead clone screening: titer. All lead clones achieved average titers of ≥5 g/L, with the two top-performing clones, A and B, reaching 7–8 g/L.

Lead clone scale-up confirmation

The performance of the lead clones, A and B, was confirmed with 14-day fed-batch assays scaled up to 3 L bioreactors. Cell growth, viability, titer, and product quality were assessed. Further assay details can be found in Table 1 (Materials and methods). With scaling to 3 L, both clones showed similarly high cell growth and viability (Figure 4), with comparable titers across scales (Figure 5).

Clone B was retested for performance consistency at a 5 L bioreactor scale. The 5 L bioreactor results were comparable to those at the 3 L scale, showing similarly strong average cell growth and viability (Figure 6) and an average titer of 8 g/L (Figure 7). N-glycan and charge variant product quality across scaling aligned with the established standards of the reference (Figures 8 and 9). Aggregation results were acceptable, with high main peaks of 93–95% across scaling (data not shown).

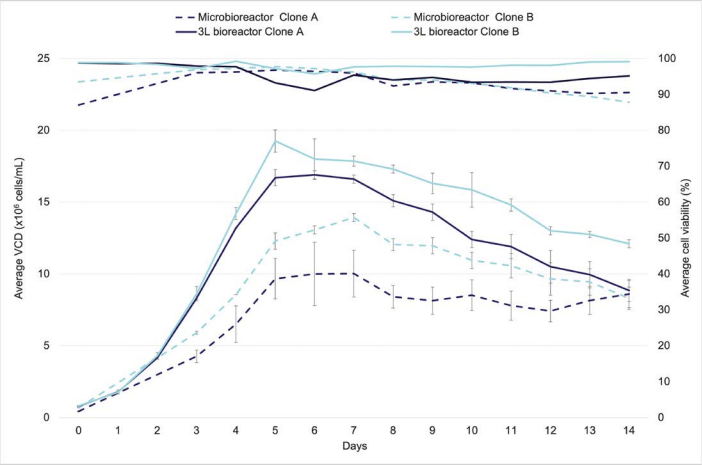

Figure 4. Scale-up 3 L confirmation: VCD and viability. At the 3 L scale, clones A and B showed similar growth profiles and peak average VCDs of 16–19 x 10^6 cells/mL, with ≥91% viability through day 14. With scaling, growth and viability overall were higher for both clones than at the microbioreactor scale. n = 3–6 for microbioreactor vessels, n = 2 for 3 L bioreactors

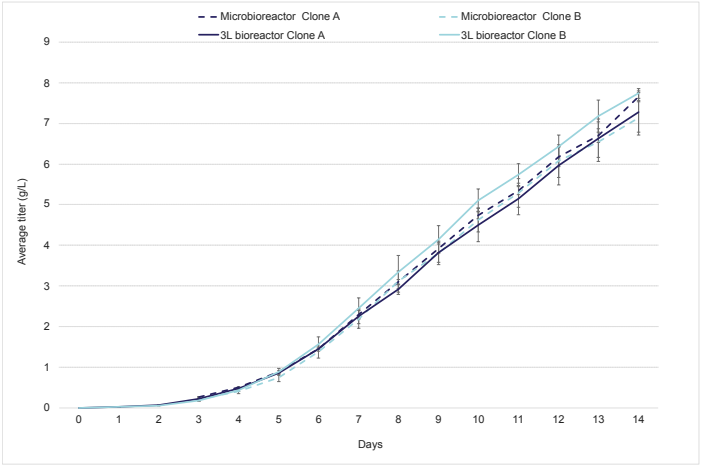

Figure 5. Scale-up 3 L confirmation: titer. Steady productivity performance across scaling was confirmed at the 3 L scale for both lead clones, with an average titer of 7.3 g/L for clone A and a slightly higher average titer of 7.7 g/L for clone B.

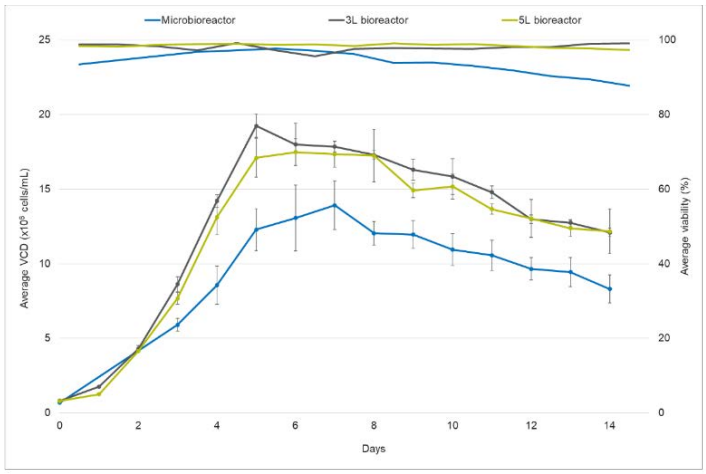

Figure 6. Consistency with scaling: VCD and viability. With additional testing at the 5 L scale, clone B showed comparable growth to the 3 L scale, with a peak average VCD of 16–19 x 10^6 cells/mL and ≥91% viability through day 14. Overall, stronger growth and viability were shown with scaling relative to the microbioreactor results. n = 3–6 for microbioreactor vessels, n = 2 for 3 L and 5 L bioreactors

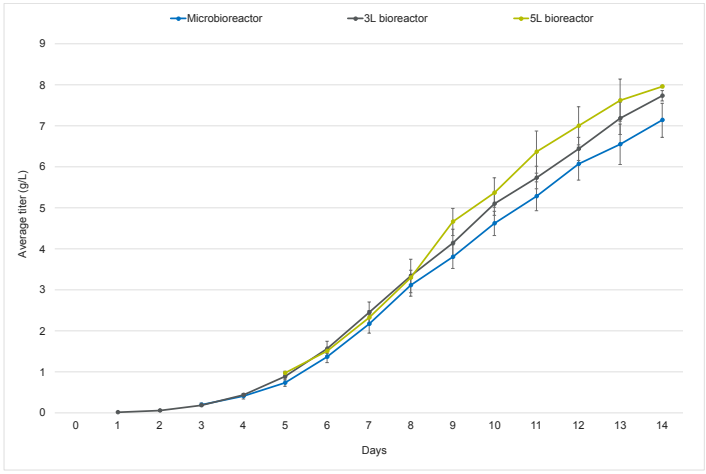

Figure 7. Consistency with scaling: titer. Clone B productivity was found to be consistently comparable to previous results, achieving an average titer of 8.0 g/L at the 5 L scale relative to 7.7 g/L at the 3 L scale. Overall, across scaling, titer results were shown to be reliably stable between 7 and 8 g/L.

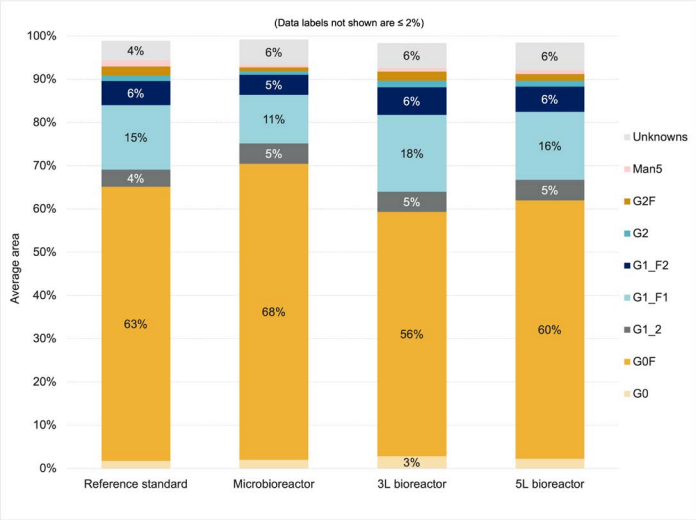

Figure 8. Consistency with scaling: N-glycans. Clone B yielded generally uniform N-glycan profiles with <10% difference in each structure from the reference standard and across scales. A slight beneficial shift toward more galactosylated forms was noted with scaling.

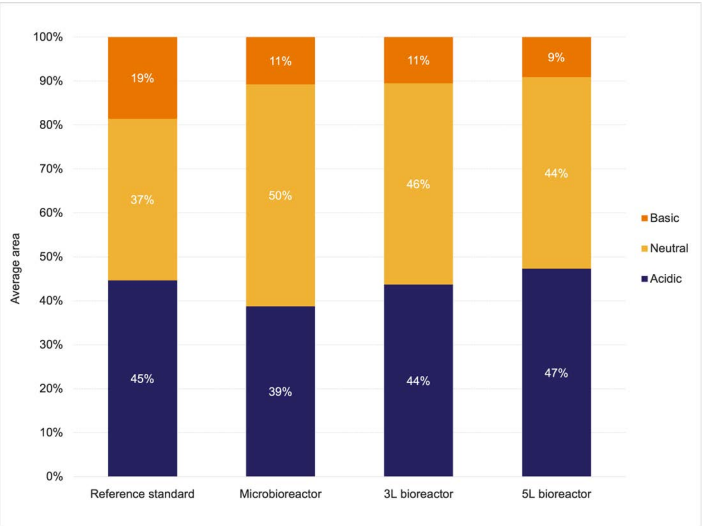

Figure 9. Consistency with scaling: charge variants. Clone B provided consistent charge variant profiles with <10% difference in each charge from the reference standard and across scales. A small beneficial shift toward the reference standard was noted with scaling.

Clone production stability

Titer performance for multiple clones was assessed up to 60 generations in passage, with the lead clones A and B evaluated further to 100 generations, using a 7-day simple (glucose-only) fed-batch assay in shake flasks. Clone stability generation tracking commenced at thaw and continued through cryo-recovery and stability assessment, with cell counts recorded at both harvest and cell seeding to monitor generations. Clones were designated as stable if they maintained ≥70% of the relative initial titer performance across the PDLs tested. Further assay details can be found in Table 1 (Materials and methods).

Acceptable stable titer production to 60 PDL was demonstrated across all 10 lead clones tested, with the top clones, A and B, showing stability through 100 PDL (Figure 10).

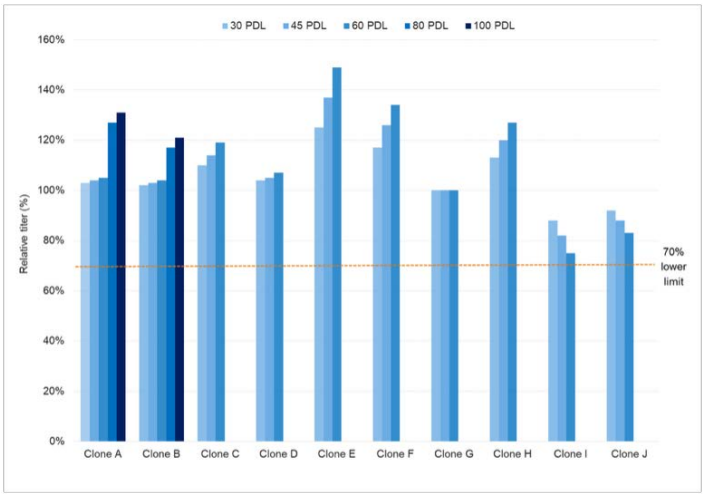

Figure 10. Stable clone production. Across the lead clones tested to a 60 PDL, all demonstrated acceptably stable titer production above the 70% lower relative titer limit. The lead clones A and B were assessed further to 100 PDL and shown to be stable at this level. n = 1 in shake flasks

Summary and discussion

Overall, the cell line development results shown demonstrate the Gibco CHOvantage GS CLD Kit supports:

- Accelerated stable mAb cell line generation and research cell banking within 14 weeks
- Reliably high titer (>7 g/L), cell growth, and viability
- Product quality consistent with the reference standard
- Confirmed dependable benchtop scalability
- Stable cell productivity up to 60 to 100 PDL

Rapid and cost-efficient delivery of mAb treatments to patients can begin by developing a stable cell line early in development with an integrated transposon-based CLD kit approach. The CHOvantage GS CLD Kit can help by offering:

- A larger pool of high-performing stable clones requiring less screening
- Process consistency and regulatory support with a cGMP-banked cell line, and a catalog chemically defined (CD) and animal origin–free (AOF) medium and feed platform with regulatory support documentation
- Efficient bioproduction scale-up with liquid, Gibco™ Advanced Granulation Technology™ (AGT™), or dry powder medium (DPM) formats for Gibco™ Efficient-Pro™ Feed Medium (+) Insulin, Feed 3, and Feed Enhancer in a range of packaging options
- Support with a detailed user guide and access to highly experienced and knowledgeable field applications and R&D scientists
- Simplified, flexible licensing with royalty-free, milestone-free commercial licensing options

For over 60 years, Gibco™ products have been helping scientists and developers move past the hurdles of biologic cell culture production. The Gibco brand offers the products and support you need to navigate the requirements of your antibody development.

References

1. Tschorn, N., Berg, K., Stitz, J. (2020). Transposon vector-mediated stable gene transfer for the accelerated establishment of recombinant mammalian cell pools allowing for high-yield production of biologics. *Biotechnology Letters,* 42(7), 1103–1112. doi.org/10.1007/s10529-020-02889-y
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Table 1. Materials and methods.

Parameter	Stable pools and clone screening	3 L lead clone confirmation scale-up	5 L lead clone confirmation scale-up	Clone stability study
Cell line	CHOvantage GS cell line			
Vessels and replicates	Ambr15™ microbioreactors (Sartorius) in triplicate vessels	3 L glass stirred-tank bioreactors in duplicate vessels	5 L glass stirred-tank bioreactors in duplicate vessels	Shake flasks, 10 clones in a singlet vessel
Medium and supplementation	Efficient-Pro Medium (+) Insulin with 4 mM L-glutamine and 1% Gibco™ Anti-clumping Agent			
Feeding strategies	Bolus feeding of 2% Efficient-Pro Feed 3 on day 2, with 3.8–4.0% Efficient-Pro Feed 3 and 0.4% Efficient-Pro Feed Enhancer daily on days 3–13	Bolus feeding of 2% Efficient-Pro Feed 3 on day 2, with 3.5% Efficient-Pro Feed 3 on days 1–13 and 0.4% Efficient-Pro Feed Enhancer daily on days 2–13	Continuous gravimetric feeding of 3.5% Efficient-Pro Feed 3 on days 1–13, with 0.4% Efficient-Pro Feed Enhancer daily bolus on days 2–13	—
Temperature	37°C, with temperature shift to 34°C on day 4			37°C
Glucose	Stable pool medium supplemented with 2.5 g/L on day 0, and clones maintained between 4 and 6 g/L	Bolus fed when <4 g/L to 6 g/L		Bolus addition of 5 g/L glucose on day 4
Seeding density	~0.7 x 10^6 viable cells/mL	0.75 x 10^6 viable cells/mL	0.75 x 10^6 viable cells/mL	0.2 x 10^6 viable cells/mL
VCD and viability	ViCELL™ BLU Cell Analyzer (Beckman Coulter)			
Titer	Cedex™ Bio HT Analyzer (Roche)	Cedex™ Bio HT Analyzer (Roche) Day 14 titers for 5 L lead clone confirmed by HPLC using Thermo Scientific™ MAbPac™ Protein A column		Cedex™ Bio HT Analyzer (Roche)
Aggregation	—	Size exclusion HPLC		—
Charge variants	—	Strong cation exchange HPLC		—
N-glycans	—	Reductive amination labeling and HILIC UPLC		—

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