

MYCENAX WhitePaper

*From Shake Flask to 50 L:
Successful Process Development
of the CHO-MK Host Cell in
Collaboration with Chitose*

About Author

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5+ years of experience in upstream development, combining practical bioreactor operation with process optimization and data-driven analysis. His work focuses on identifying critical parameters through thoughtful experimental design and integrating statistical insight with hands-on process understanding to enable robust and scalable upstream performance.

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16+ years of experience in the CRO and CDMO industry, leading Mycenax's bioprocess innovation initiatives in cell line, upstream and downstream process optimization to advance protein production efficiency and scalability.

About Mycenax Mycenax is a dedicated CDMO specializing in process development and cGMP manufacturing of biologics and biosimilars. We provide integrated DNA-to-Drug Product services for mAbs, ADCs, plasmid DNA, cell therapies, vaccines, and more through advanced platforms and PIC/S GMP-compliant facilities. Our GMP site is approved by EMA, MHRA, PMDA, Health Canada and MFDS, and has successfully passed over 70 global client audits, ensuring the highest standards of quality and compliance.



Executive Summary

Mycenax Biotech collaborated with Chitose Laboratory Corp., a Japan-based biotech company renowned for developing the innovative CHO-MK host cell line.

Through a joint effort, Mycenax successfully transferred Chitose's shake-flask culture conditions, established a robust 2 L bioreactor process, and further scaled it up to 50 L, achieving consistent performance and productivity.

This milestone demonstrates our capability to efficiently transform emerging cell line technologies into scalable, GMP-ready manufacturing processes—bringing innovation into scalable manufacturing.

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 **CHITOSE**

CHO-MK and the Future of Process Intensification

The manufacturing of therapeutic proteins is evolving toward **process intensification**, driven by the industry's pursuit of **higher production efficiency** through shorter production cycles, reduced facility footprint, and more efficient use of time and resources.

In upstream processing, these goals are typically achieved by optimizing culture media to enhance cell productivity and growth density, together with adopting intensified fed-batch or perfusion strategies to maximize volumetric yield.

In recent years, the CHO-MK cell line, developed by Chitose Laboratory Corp., has gained strong attention as a promising next-generation host for intensified bioprocessing. With its rapid growth rate and high specific productivity, CHO-MK can reach comparable or higher titers in roughly half the culture time of conventional fed-batch processes (**Figure 1**). This enables shorter batch turnaround and lower manufacturing cost — all well aligned with the principles of process intensification.

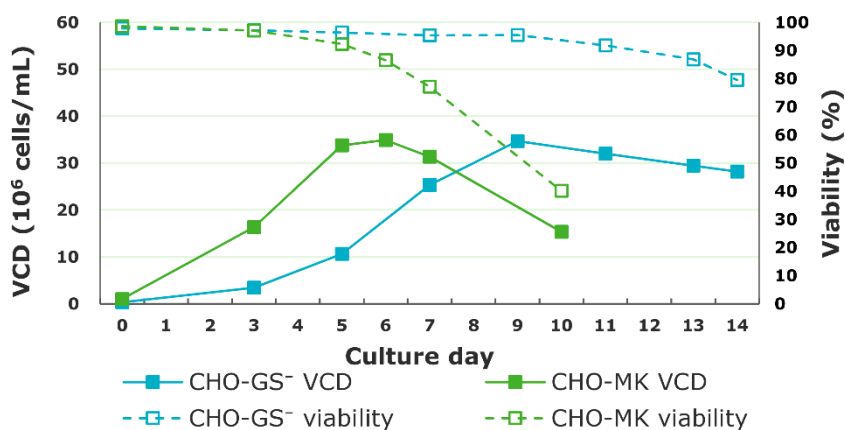


Figure 1. Compared with conventional CHO cells (represented by CHO-GS⁻) operated in a 14-day fed-batch culture, CHO-MK completed production within approximately 7 days with comparable or even higher final titers (data not shown).

To explore this potential, we collaborated with Chitose to carry out a technology transfer and process development project using a monoclonal antibody as a model molecule. CHO-MK's highly proliferative nature required careful control of oxygen and seed-train conditions. Through deliberate process design and systematic optimization, we successfully translated Chitose's shake-flask culture conditions into a bioreactor-based process, established a robust 2 L reactor process, and further scaled it up to 50 L, achieving comparable cell growth profiles and productivity.

The following sections demonstrate the stepwise progression and results across three key stages — technology transfer, process development, and scale-up — highlighting how we enabled the successful implementation of the CHO-MK platform.



Translating Potential into Scalable Performance

1. Technology Transfer

We initiated a comprehensive technology transfer process based on the data package provided by Chitose. Using the supplied shake-flask parameters (**Table 1**), the CHO-MK culture conditions were carefully reconstructed in our laboratory to verify the reproducibility of the original results.

Table 1. Shake flask culture settings used for CHO-MK reproducibility testing

Parameter	Setting
Scale	125 mL
Shaking speed (rpm)	150
Working volume (mL)	22
Temperature(°C)	37
CO ₂ (%)	5
Initial cell density (cells/mL)	1 x 10 ⁶

During process observation, CHO-MK cells were found to exhibit relatively high oxygen demand. By adjusting the shaking speed to enhance gas transfer, the cells exhibited normal and rapid growth.

The reproducibility test demonstrated that both the transferred and original CHO-MK cultures achieved comparable performance, with peak viable cell density (VCD) reaching 3.5×10^7 cells/mL and final titer around 8 g/L (**Figure 2**). These results confirmed the successful replication of Chitose's original process at Mycenax and established a solid baseline for subsequent bioreactor development.

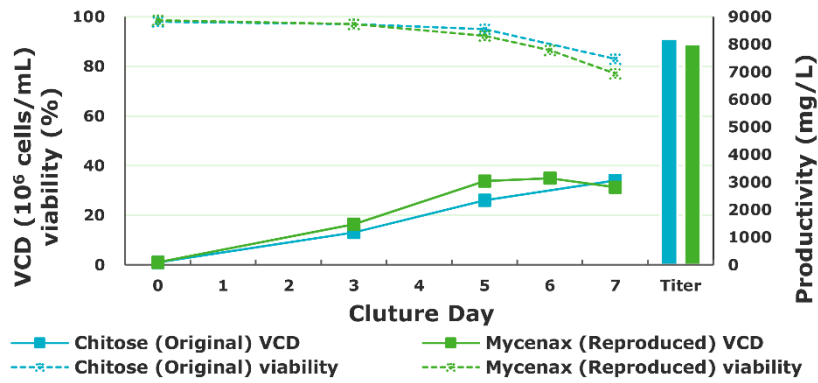


Figure 2. Reproducibility of CHO-MK shake-flask culture between Chitose and Mycenax

2. 2 L Bioreactor Process Development

The bioreactor parameters were initially established based on the results of the shake-flask study and subsequently refined through empirical optimization (**Table 2**). The seed-train condition was customized to accommodate CHO-MK's highly proliferative nature. Feeding regimens, as well as pH and DO controls, were fine-tuned to support active metabolism and maintain culture stability throughout the production phase. In addition, the agitation setting was adjusted to ensure sufficient oxygen transfer and homogeneous nutrient distribution.

Table 2. Bioreactor operating parameters for CHO-MK process development

Parameter	Set point / range
pH	6.85 – 7.40
DO (%)	60
Agitation (rpm)	300 - 430
Temperature(°C)	37
Seeding density (cells/mL)	1 x 10 ⁶

The resulting 2 L bioreactor culture achieved peak viable cell density of approximately 4×10^7 cells/mL and a final titer close to 8 g/L, comparable to the shake-flask culture results (**Figure 3**). These outcomes demonstrated that the process design and control strategy effectively translated the flask-based system into a scalable, well-controlled bioreactor process suitable for further scale-up.

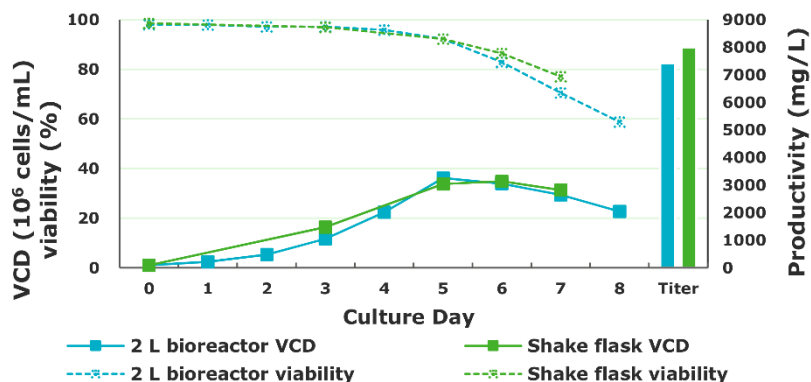


Figure 3. Comparison of CHO-MK growth performance between shake flask and 2 L bioreactor cultures

3. 50 L Scale-Up and Validation

The optimized 2 L bioreactor process was directly scaled up to a 50 L bioreactor at Mycenax's pilot facility. Scale-up parameters were converted using Mycenax's internal scale-up platform, which maintains a constant power input per volume (P/V) between vessels to preserve mixing and gas-transfer dynamics across different reactor sizes. Through systematic control of gas transfer and feed supplementation, process consistency was successfully maintained during the scale-up run. The cell growth profile, viability, and productivity in the 50 L bioreactor were almost identical to those observed at the 2 L scale (**Figure 4**).

These results demonstrate excellent process scalability and robustness, confirming the reliability of Mycenax's bioreactor design and scale-up strategy for CHO-MK cell-based production.

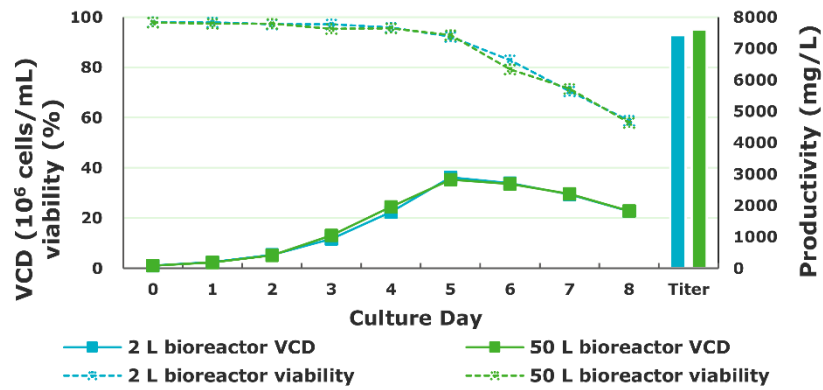


Figure 4. Comparison of CHO-MK performance between 2 L and 50 L bioreactor cultures

Conclusion

From shake-flask reproduction to 2 L bioreactor development and 50 L scale-up, the joint project successfully demonstrated the seamless translation of CHO-MK cell technology into a scalable, well-controlled manufacturing process. Each stage validated Mycenax's capability to reproduce, optimize, and expand process performance while maintaining consistency in cell growth profile and productivity.

The collaboration between Chitose and Mycenax illustrates how innovative cell technologies can be efficiently industrialized through an experienced CDMO partner. By combining Mycenax's deep expertise in mammalian process development with Chitose's next-generation CHO-MK cell line, the project achieved both technical success and strategic validation.

With this foundation, Mycenax stands ready to support further scale-up, process optimization, and cGMP manufacturing for global clients adopting this powerful new host system.



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