Making the Medicines of Tomorrow

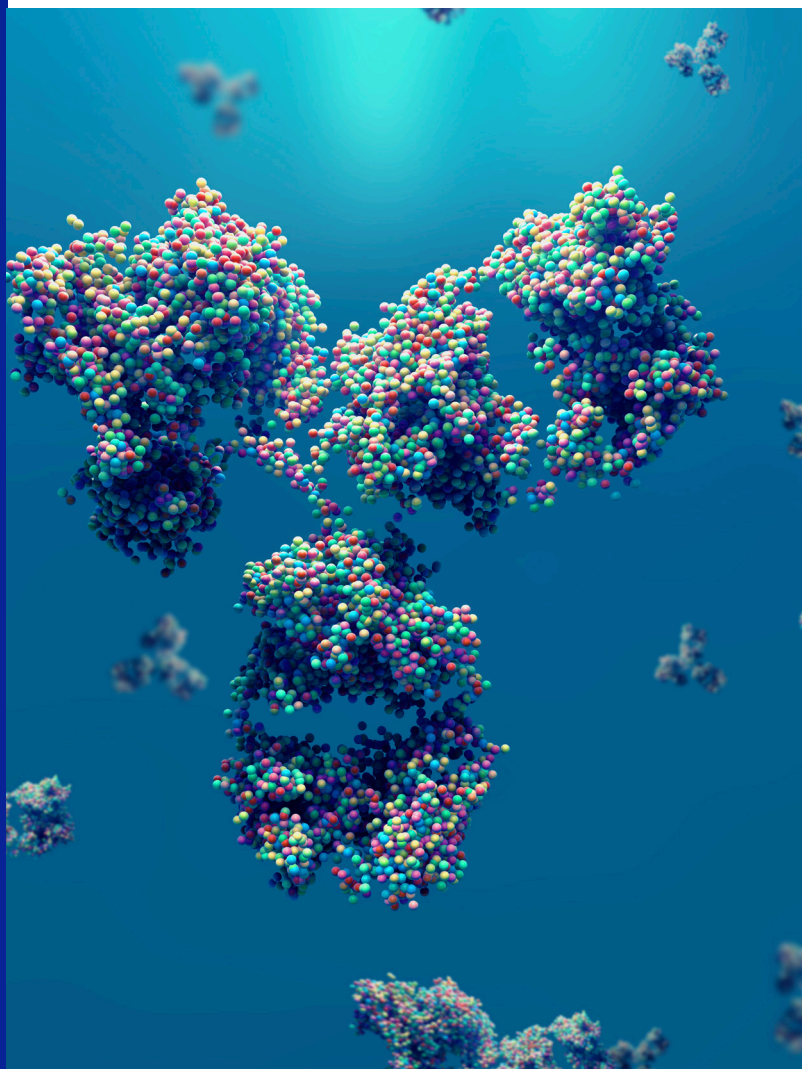
Lonza
Specialized Modalities

Therapeutic protein production made simple

Overcome CHO cell culture challenges and maximize productivity with an easy-to-use, high-performance fed-batch media system

The protein therapeutics market is growing at pace and is estimated to reach a value of more than $566 billion by 2030. To compete in this space, biopharmaceutical companies must be able to produce large amounts of high-quality recombinant proteins, quickly. Achieving this goal, however, hinges on companies selecting an optimal expression system and the best accompanying cell culture media and feeds.

In this whitepaper, we provide an overview of Chinese Hamster Ovary (CHO) cells as the go-to platform for therapeutic protein production, discuss the challenges manufacturers have faced (and continue to face) with CHO cell culture media systems, and introduce **a complete CHO cell culture system that overcomes these challenges to deliver maximum performance and ease-of-use**.



CHO Cells: The go-to platform for therapeutic protein production

Companies looking to develop and manufacture recombinant protein therapies have a range of expression systems to choose from — namely insect, bacterial, yeast, and mammalian systems. Making the right selection involves carefully considering several factors, including ease of use, total cost of ownership (the direct and indirect costs of the system throughout its lifespan), generation time, yield, and post-translational modifications required for the recombinant protein in question.

The most prevalent expression system for therapeutic protein production, by far, is the CHO system. The reasons are numerous: industrial CHO cell lines have been adapted so that they can be grown in suspension, enabling volumetric scalability; these same cell lines exhibit highdensity cell growth and highly stable protein expression; and they also produce proteins that are therapeutically active (since CHO cells can properly fold complex proteins and perform human-like post-translational modifications). In addition, an extensive glossary of protocols, built up over many years, is available for conducting CHO cell culture across a range of scales. And, most importantly, CHO systems have a long history of regulatory acceptance and safety.

A variety of CHO cell lines are available on the market for therapeutic protein production, each with different relative advantages and disadvantages. Some of the most common are noted below:

- **CHO-K1 cells** – Known for their robust, high-productivity growth, and extensively utilized in biotechnological applications. However, they are adherent and require a solid surface for growth, and are highly susceptible to instability over time
- CHO-GS cells – CHO-GS cells are modified to include a knock-out of the glutamine synthetase gene for metabolic selection, eliminating evasion in the selection process and thus increasing efficiency in isolating high-producing cell lines. Although efficient in producing stable, high-productivity clones, CHO-GS cells have very specific media requirements
- **CHO-S cells** – These cells are adapted for suspension culture and can achieve high cell densities, making them suitable for large-scale protein production. However, they have very particular growth medium requirements and may yield lower productivity
- **CHO-DG44 cells** – Derived from the original CHO cell line through deletion of the dihydrofolate reductase (DHFR) gene, CHO-DG44 cells enable DHFR-mediated gene amplification, which is useful in selecting recombinant clones. However, these cells require supplementation with hypoxanthine and thymidine (HT) for growth, which can increase the complexity of media preparation
- **CHO-M cells** – This cell line is optimized for metabolic efficiency and high-density cell growth in suspension culture, although media optimization may be required for specific proteins

Together, these different CHO cell lines play a crucial role in therapeutic protein production.

CHO Cell Culture Media: Historical and current challenges

Despite the popularity of CHO cells for protein production, culturing them has historically been — and in many cases can still be — difficult, owing to several challenges associated with CHO cell culture media systems.

Reliance on fetal bovine serum

Perhaps the most significant of the historical challenges relates to CHO cell culture media not being chemically defined. For example, for much of their history, CHO cell culture media systems have relied on the addition of fetal bovine serum (FBS) to promote cell growth. FBS, however, is a 'black box' containing a host of unidentified and undefined components, including growth inhibitors, which can compromise cell growth. Since FBS is animal-derived, it can also exhibit substantial batch-to-batch variability, leading to inconsistent cell culture performance and, thus, difficulties scaling with and between R&D, pilot, and production scales. Due to the potential presence of harmful contaminants in FBS, drug developers and manufacturers must also conduct time-and resource-intensive safety testing of both FBS and the bulk drug substance produced using FBS. Finally, depending on its cost and availability, FBS may be unaffordable or too risky for larger-scale cell culture.

Eliminating FBS in favor of growth factors

Early attempts to overcome the challenges of FBS included replacing FBS with defined amounts of specific growth factors. While this eliminated components such as inhibitors and helped mitigate some of the variation associated with FBS, cell culture challenges persisted; drug developers and manufacturers still saw considerable batch-to-batch variability since growth factors are animal derived, and therefore experienced robustness issues when scaling. Growth factors were also cost prohibitive for larger scale cell culture, further adding to scaling challenges.

The industry then moved to cell culture media formulations using non-animal bio-lysates. However, while animal-free, these components were not chemically defined, and so still posed variability issues, especially when culturing at scale.

The Gold Standard: Non-animal origin, Chemically-defined media

Eventually, the industry arrived at what is today considered the gold standard of cell culture media — media that are non-animal origin and fully chemically defined. Such media are entirely free of animal derived ingredients and contain only raw materials of known chemical composition, quantity, and quality. As a result, companies benefit from more control over process inputs, greater batch-to-batch consistency, and greater process robustness at scale.

A lingering obstacle: Media prep complexity
Although animal component-free, chemically defined media systems have been developed to yield high cell growth and titers — while maintaining high product quality — they have traditionally been complex to prepare and use, consisting of multiple bases and feeds with solubility and stability issues.

These issues result in complex multistep processes for media preparation and use, restricting media pre-preparation and extending media preparation times, which can have significant impacts on the cost and efficiency of large-scale therapeutic protein manufacturing processes.

Scalability struggles
Another significant industry challenge — observed both historically and today — is the scalability of CHO cell culture media. More specifically, as developers move from lab-scale vessels to pilot- and production-scale bioreactors, it is common to observe inconsistent media performance, leading to lower product yields and suboptimal product quality. This is partly due to the different properties and parameters of different sized bioreactors (height : diameter ratios, for example), and because the chemical make-up of many cell culture media systems can behave differently at scale. While achieving consistent cell culture outcomes across scales requires extensive optimization of many interrelated process parameters, having a robust cell culture media system can significantly reduce the complexity and effort of scaling.

Overcoming the complexity of CHO cell culture, without sacrificing

Performance
To address the above-mentioned media challenges, Lonza has developed the TheraPRO® CHO Media System — a complete, animal component-free, and chemically defined CHO cell culture media system, optimized for the Lonza CHOK1SV GS-KO® cell line, that delivers **exceptional ease-of use and superior performance.**

The system comprises four components which together constitute an end-to-end media solution for your bioprocess manufacturing needs.

- The TheraPRO® CHO Cloning Medium (Liquid — ready to use)
- The TheraPRO® CHO Expansion Medium (Liquid — ready to use)
- The TheraPRO® CHO Production Medium (Powder — ready to dissolve)
- The TheraPRO® CHO Feed (Powder — ready to dissolve)

In addition, TheraPRO® CHO Media System customers get access to Lonza's world-renowned technical support for easy implementation and swift troubleshooting to ensure the best results.

Reduced prep time and complexity, fewer mistakes

With the new media system, preparation is simple, requires fewer steps, and involves minimal time and effort:

- The cloning medium comes as a convenient, ready-to-use liquid solution, **demanding no preparation time**
- The expansion component comes as a single, ready-to-use bottled formulation, **demanding no preparation time**
- The production media and feeds components each comprise a single powder, requiring just **1 hour and 1.5 hours of preparation time for the media and feeds, respectively**

Overall, the total maximum media and feed system prep time, from revival and subculture through to production and feeds, is **2.5 hours**.

Due to the system's simplicity and minimal preparation time, end-users can expect **reduced operational costs**, and **fewer operator errors**. What's more, end users can benefit from **simple purchasing**, too, helping save further time and resources.

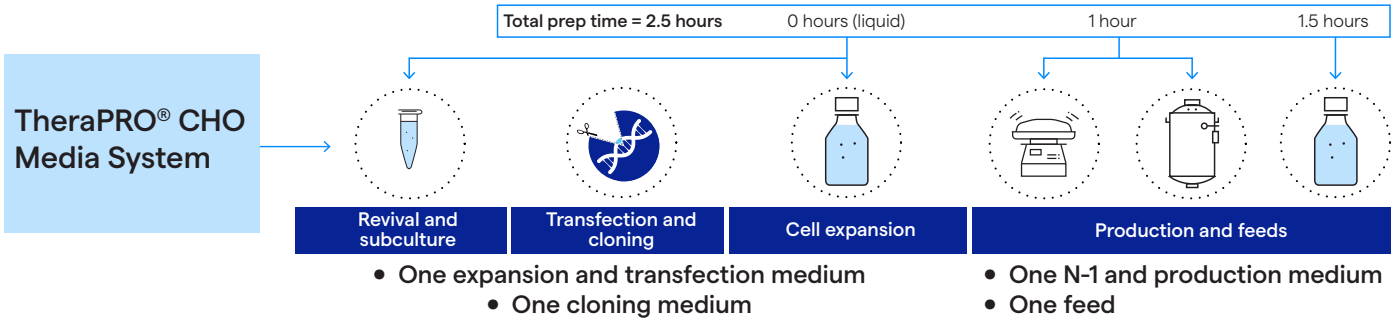


Figure 1.
Media preparation time for the TheraPRO® CHO Media System compared to previous media systems.

Exceptional performance

The new system, in addition to offering simplicity and ease-of-use, is flexible and delivers high levels of performance.

Cloning: Compatibility with different cloning technologies

The TheraPRO® CHO Media System is compatible with different cloning techniques, including fluorescence-activated cell sorting (FACS) and optofluidics-based methods (Table 1). *Table 1: Recommended TheraPRO CHO® Media System components for different cell cloning methods.*

Cloning methods	Recommended media during cloning	Recommended media for recovery post cloning
FACS sorting	TheraPRO® CHO Cloning Media	TheraPRO® CHO Cloning Media
Optofluidics based systems	TheraPRO® CHO Expression Media supplemented with HEPES	TheraPRO® CHO Expression Media

Expansion: Good post-transfectionc cell recovery, high viable cell concentrations and viability

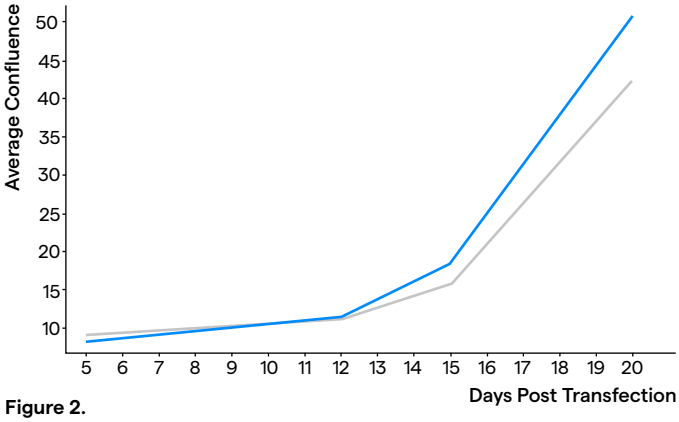


Figure 2.
Cell recovery post-transfection. Average confluence measured in two different lots of TheraPRO® CHO Expansion Medium, using imaging on 96-well plates.

With the TheraPRO® CHO Expansion Medium, good average confluence (>40%) can be achieved in under three weeks (figure 2), indicating good cell recovery post-transfection, and **enabling short cell line construction timelines.**

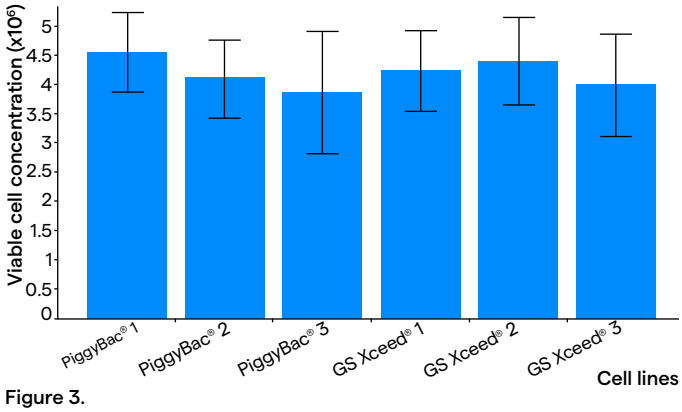


Figure 3.
Average viable cell concentration using TheraPRO® CHO Expansion Media with six different cell lines (n=18).

After investigating the performance of the TheraPRO® CHO Expansion Medium in six different cell lines, Lonza also observed a **consistently high viable cell concentration and viability,** which was independent of the cell line used, highlighting the system's high performance and robustness across cell lines.

Production: Superior viable cell concentration, pure and high-quality proteins

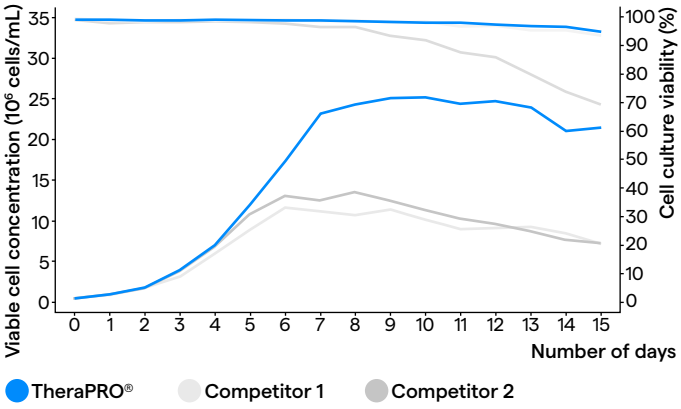


Figure 4.
Viable cell concentration and viability in cultures using Lonza's TheraPRO® Production Medium and Feed versus competitor media

Lonza compared the viable cell concentration and viability over time when using TheraPRO® CHO Production Medium and two other competitor media in 10 L bioreactors.

As seen in figure 4, growth profiles diverged at ~5 days post-inoculation. **The culture using the TheraPRO® Production Medium and Feed went on to peak at a viable cell density of 28 x 10^6 cells/mL and stayed high, at or above 24 x 10^6 cells/mL, throughout the time course.** Competitor media, on the other hand, peaked at just 12 – 15 x 10^6 cells/mL (a typical bioreactor yield), and gradually declined thereafter. The cell culture viability when using the TheraPRO® CHO Medium and Feed also stayed above 90% for the duration of the 15-day run, unlike with competitor systems. By enabling such high cell culture viability and viable cell concentrations, the TheraPRO® CHO Production Medium and Feed can help support high productivity and elevated product titers.

Monomer and aggregate percentages using TheraPRO® CHO production medium and feed

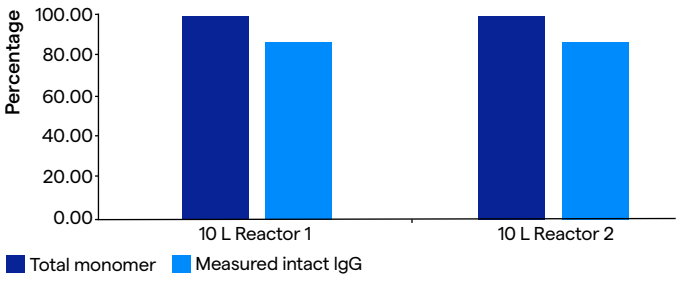


Figure 5.
Monomer and aggregate percentages in two different 10 L bioreactor cultures using Lonza's TheraPRO® Production Medium and Feed.

The TheraPRO® Production Medium and Feed also enables consistent production of pure, high-quality proteins. In the same experiment, the Lonza TheraPRO® Production Medium and Feed delivered **high monomer percentages across the bioreactor duplicates** (>99.7% in both reactors), exceeding the industry acceptance criteria of 95%. Moreover, **the percentage of intact IgG also remained high (87%) in both reactors,** which is representative of high-purity pro-duction of the IgG isotype (which does not typically exceed ~85% purity), indicating no significant amounts of low or high molecular weight species.

Scalability

Lonza has tested the performance and behavior of the TheraPRO® CHO Media System extensively across different scales and cell culture types — from plates, flasks, scaled down bioreactors, stirred tanks, and single-use bioreactors, to production scale vessels. **Viable cell concentration, cell culture viability, final product titer, and product quality attributes remain consistent from 15 mL right up to 20,000 L bioproduction.** By offering exceptional, scalable performance, the TheraPRO® CHO Media System can help speed and de-risk scaling for a faster, more cost-effective route to market.

For a deeper dive into the scalability benefits of the TheraPRO® CHO Media System, complete with supporting data, download our free white paper, Scalable Therapeutic Protein Production Simplified.

The path to simple, high performance recombinant protein production

CHO cells have long been considered the optimal expression system for commercial therapeutic protein produc-tion. However, CHO cell culture systems have presened many obstacles for biopharmaceutical companies — variability owing to the use of animal-derived components in older formulations, a lack of robustness that can hin-der scaling, and more recently, drawn-out timelines and increased risks of operator error due to complex media preparation protocols.

The TheraPRO® CHO Media System eliminates these obstacles, delivering a complete solution that offers unprecedented ease-of-use and scalability, coupled with high performance and product quality.

To find out more about the TheraPRO® CHO Media System, reach out to one of our bioproduction experts today.

To enquire about Lonza's GS Gene Expression System® which enables access to the proprietary CHOK1SV GS-KO® cell line, in addition to optimized vectors, technologies and personalized technical support, contact licensing@lonza.com.

Contact us

North America

Customer Service: +1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: +1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe

Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +49 221 99199 400
scientific.support.eu@lonza.com

International

Contact your local Lonza distributor
Customer Service: +1 301 898 7025
scientific.support@lonza.com

Lonza Walkersville, Inc. – Walkersville, MD 21793

Learn more.

ME-SP001 07/25

bioscience.lonza.com
lonza.com/therapro