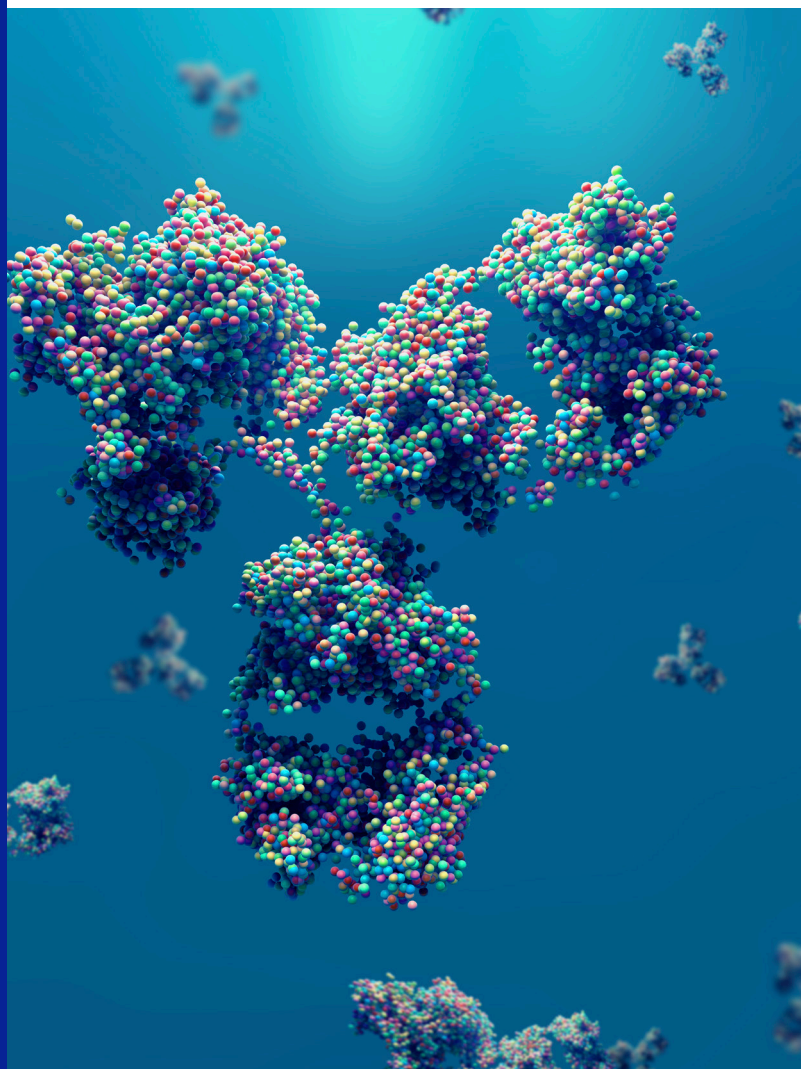
Making the Medicines of Tomorrow

Lonza
Specialized Modalities

Scalable therapeutic protein production, simplified

The challenges of bioproduction scale-up and how robust media and feeds can help overcome them

Protein therapies have changed the way that we treat a vast range of diseases and conditions, from cancers to autoimmune conditions. Understandably, the market for such therapies is large and continues to grow at pace — in 2023 alone, the global protein therapeutics market was worth ~$340 billion and is estimated to reach a value of ~$605 billion by 2033.[1]



However, bringing a recombinant protein therapy to market is not easy; it hinges, in part, on effectively scaling a cell culture production process, which can be complex, time-consuming, and costly, making it a significant stumbling block for many companies. Scaling up mammalian cell culture (such as Chinese hamster ovary (CHO) cell culture) for protein production presents several challenges for small to mid-size companies, including maintaining consistency and reproducibility of product quality, due to differences

in equipment and operational parameters across scales. Limited resources and technical expertise in such companies also make extensive process optimization costly and time-consuming. Ensuring regulatory compliance adds further complexity, as does the need for suitable scale-up equipment and infrastructure investments. To overcome these hurdles, companies must use optimized media, advanced bioreactor technologies, and quality-by-design methods.In this whitepaper, we highlight the challenges of mammalian cell culture scale up, discuss the parameters that companies must consider, and introduce a robust and scalable high-performance CHO cell culture media system that can help simplify the path to scaled therapeutic protein production.

A range of cell culture scales are used in the development of a therapeutic protein. For example, for practical purposes, companies typically use smaller-scale cell culture volumes (250 mL – 2 L) to explore and optimize an array of process parameters and create a suitable production process — one that is stable, robust, and effective (deliver-ing high cell growth and viability, and producing proteins of the right quality). However, to cost-effectively manufacture large amounts of therapeutic proteins, companies must move from small lab-scale vessels to bioreactors that can exceed 30,000 L.

Crucially, though, cell culture processes can behave very differently in larger, scaled-up vessels relative to smaller vessels, potentially leading to significantly lower product yields and suboptimal product quality. In such a scenario, companies can either settle for reduced profit margins as a result of the poorer-than-anticipated product yields, or conduct process development and optimization in large-scale reactor vessels. The latter is complex and challenging, however; given the relatively long time scales of mammali-an cell culture, the number and complexity of parameters that must be optimized, the process of preparing large volumes of media, and the access to large-scale bioreactor facilities that's required, the process can be long and very expensive. In practice, companies can rarely conduct more than 1 – 2 bioreactor runs per month.

In the best-case scenario, when scaled cell culture fails to deliver to the expected level of performance, companies face a more expensive and longer route to market. But, in the worst case, where process development in large scale vessels is not economically viable, companies may need to halt their development program altogether. Not only does this have a huge financial cost for the developer, but it also has a real-world human cost too, with patients potentially denied life-changing novel therapies.

The scale-up challenges don't end there. Therapeutic proteins produced at larger scales are often destined for clinical trials or release to the market, so they must meet stringent quality and safety requirements. To do so, companies must ensure they can secure a reliable supply of raw materials that have been thoroughly tested and screened, and proven to be of the highest quality, which can be difficult at large manufacturing scales.

Without high-quality raw materials, the risk of contamination events increases, companies benefit from more control over process inputs, greater batch-to-batch consistency, and greater process robustness at scale. potentially interrupting manufacturing schedules, or even lead to a breach of regulations, which can have significant financial and reputational consequences.

The many parameters impacting Mammalian Cell Culture process scalability

Many companies struggle with scale-up because they lack the knowledge of the bioreactor properties and operating parameters that can impact cell culture process conditions, and how the chemical makeup of cell culture media can behave differently at scale.

Naturally, if companies do not know the factors that will change (and how they will change) as they scale their cell culture, they will be less able to predict cell culture performance (or its potential failure) in larger vessels.

Bioreactor operating parameters

Bioreactor operating parameters have profound impacts on cell culture process conditions and are a crucial consideration when it comes to bioprocess scale-up. They can be divided into two categories: scale-independent parameters and scale-dependent parameters.

Scale-independent parameters are those that do not depend on bioreactor volume, and thus are easy to keep constant when scaling a cell culture process — parameters such as temperature, pH, dissolved oxygen, nutrient concentrations, media composition, and feed regimens. Typically, companies explore and optimize these parame-ters during process development at R&D scale.

Scale-dependent parameters, on the other hand, do depend on the bioreactor volume and cannot all be kept constant as you scale. These parameters include:

- Mixing time. Mixing time is the time required to achieve cell culture medium homogeneity after adding a substance or a perturbation. Efficient mixing ensures that cells are uniformly exposed to nutrients, dissolved gases, and temperature conditions. Shorter mixing times prevent the formation of concentration gradients, leading to a more consistent cell culture environment.
- Impeller tip speed. In stirred-tank bioreactors, impellers agitate the cell culture medium to ensure proper mixing and homogeneity. Impeller tip speed is simply the linear speed of the outermost edge of the impeller blades. The higher the impeller tip speed, the faster the mixing. However, higher speeds can cause shear stress which can damage delicate mammalian cells.
- Gas mass transfer coefficient (kLa). This is a measure of the efficiency of oxygen (or another gas) transfer from the gas phase into the liquid phase in a bioreactor. A higher kLa value indicates more efficient gas transfer, which is crucial for maintaining adequate oxygen levels in high-density cultures.
- Mixing power input per unit volume (P/V). P/V is a measure of how much energy is transferred from the stirrer to the liquid. P/V influences culture mixing, oxygen transfer, and shear stress.
- Reynolds number (Re). The Reynolds number is a dimensionless measure of the flow regime within a bioreac-tor (laminar or turbulent). Reynolds numbers >4,000 typically indicate turbulent flow, which enhances mixing and gas transfer. However, very high values can lead to excessive shear stress.
- Volumetric gas flow rate per unit volume (VVM). VVM is a measure of the rate at which gas is introduced into the bioreactor per unit volume of cell culture medium. Adequate gas flow rates are essential for maintaining optimal oxygen levels and removing excess CO_2. However, gas flow rates that are too high can lead to foaming and shear stress, while rates that are too low can result in insufficient oxygen supply to cells, and CO_2 buildup.

These scale-dependent parameters cannot be kept constant for several reasons. One is that scale-dependent parameters are highly interdependent. For example, to keep the mixing time constant, companies will need to drastically increase impeller tip speed as bioreactor volume increases. In addition, the technical limitations of bioreactors — and the fragility of mammalian cells — make it impossible to maintain certain parameters across reaction vessel sizes. Mammalian cell susceptibility to shear stress, for instance, prohibits the aggressive mixing required to maintain a constant mixing time in larger vessels.

In light of this difficulty, the typical approach to bioprocess scale-up is to keep all scale-independent parameters, and bioreactor geometry, the same while prioritizing a single or several scale-dependent variables to keep constant. Constant P/V is one of the most commonly used scaling criteria, given that this value provides a good indicator of cell culture homogenization, mixing, and shear stress. kLA is also a popular scaling criterion.

Whichever approach companies settle on for scale-up, longer mixing times are a significant and inevitable challenge as bioreactor volumes increase, jumping from ~10 seconds in lab-scale bioreactors to ~120 – 360 seconds in 12,000 L stirred-tank bioreactors.[2,3] This is important, since poor mixing can lead to inhomogeneities in pH, dissolved O_2, partial pressure of CO_2 (pCO_2), and nutri-ent concentration throughout vessels, exposing cells to different microenvironments for varying lengths of time. Crucially, exposure to these environmental fluctuations can significantly impact cell culture performance. Indeed, several studies have investigated the effects of inhomoge-neities on mammalian cell culture in scaled-down bioreac-tor systems, demonstrating altered metabolism, reduced cell growth and productivity, increased by-product accu-mulation, and altered product quality attributes (such as glycosylation patterns).[4, 5, 6, 7, 8, 9]

Cell culture media considerations

Bioreactor operating parameters are not the only variables companies must consider when attempting to scale their cell culture processes. The chemical makeup of cell culture media and feeds can profoundly impact whether companies can replicate cell culture performance in larger bioreactors, too. For example, with some media and feeds, the small changes in temperature, aeration, and osmolality that are common in large-scale bioreactors can impact media performance such that cell growth and productivity are significantly reduced.

In addition, some cell culture media are susceptible to nutrient depletion. In stirred-tank bioreactors, shear stress is highest at the bottom of the vessel, leading to increased cell stress and, thus, increased localized consumption of specific nutrients. If the cell culture media system cannot accommodate increased consumption of these nutrients, nutrient levels may fall below physiologically optimal levels, resulting in reduced cell growth and viability, and altered product quality. As such, it is critical that companies select a robust cell culture media — one that can tolerate the small environmental changes common in larger-scale bioreactors, and maintain adequate nutrient supply in the presence of common or anticipated nutrient depletion patterns.

Simplifying scale-up with a highly scalable CHO Cell Culture Media System

To help simplify and de-risk bioproduction process scale-up, Lonza has developed the TheraPRO® CHO Production Medium and Feed — an easy-to-use, stable, high-perfor-mance medium and feed optimized for the CHOK1SV GS-KO® cell line and suitable for any production scale.

The TheraPRO® CHO Production Medium and Feed are part of Lonza's TheraPRO® CHO Media System — a complete, fully non-animal origin, and chemically defined CHO cell culture media system that delivers exceptional ease of use and performance. In addition to the TheraPRO® CHO Production Medium and Feed, the system comprises the TheraPRO® CHO Cloning Medium and TheraPRO® CHO Expansion Medium, providing an end-to-end media solution for your bioprocess manufacturing needs.

For more information and data about the performance of the system as a whole, and to discover why Chinese hamster ovary (CHO) cells are the most prevalent express system for therapeutic protein production, download our free whitepaper, "Therapeutic Protein Production Made Simple".

The same high performance across scales

The Lonza TheraPRO® CHO Production Medium and Feed has been extensively tested in vessels ranging from 15 mL shake flasks to 200 L bioreactors, with its performance also modelled in larger reaction vessels up to 20,000 L. Exper-imental results demonstrate that the medium and feed offers the same excellent performance, regardless of scale:

Viable Cell Concentration and Cell Culture Viability across different scales

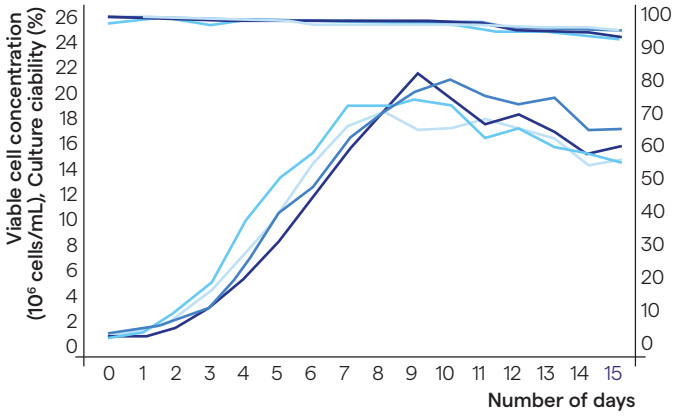


Figure 1.
Graph showing viable cell concentration (x10^6 cells/mL) and cell viability over a 15-day run using Lonza's TheraPRO® CHO Production Medium and Feed in cell culture vessels ranging from 100 mL to 200 L in volume.

While some CHO cell culture media systems can support a high cell density at smaller scales, they can struggle to maintain this performance in larger vessels. However, experiments show that Lonza TheraPRO® CHO Production Medium and Feed supports consistently high viable cell concentrations at 100 mL, 10 L, 50 L, and 200 L scales.

As illustrated in Figure 1, the cell cultures maintain similar growth rates across different scales while achieving viable cell concentrations exceeding 20 x10^6 cells/mL. Our experiments also show that cell culture scale does not impact the high cell viability supported by the TheraPRO® CHO Production Medium and Feed; in all cultivation vessels tested, cell viability remained >90% for the 15-day run. This high level of consistency and viable cell density is essential for optimizing productivity in bioprocessing applications.

Lactate concentration across different scales

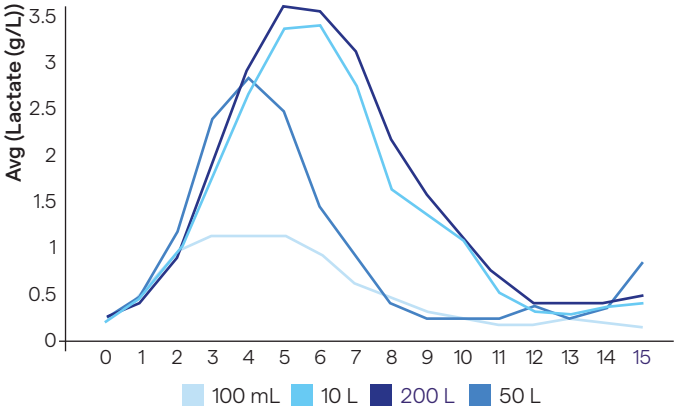


Figure 2.
Graph showing lactate concentration (g/L) over a 15-day run using Lonza's TheraPRO® CHO Production Medium in cell culture vessels ranging from 100 mL to 200 L in volume.

Lactate is a key waste product in mammalian cell culture, where excess accumulation can detrimentally impact cell culture performance. Generally, less than 4 g/L is considered acceptable in CHO cell culture. In our experiments, the TheraPRO® CHO Production Medium and Feed maintained lactate levels below 4 g/L in every cell culture scale tested (Figure 3).

Product titer across different scales

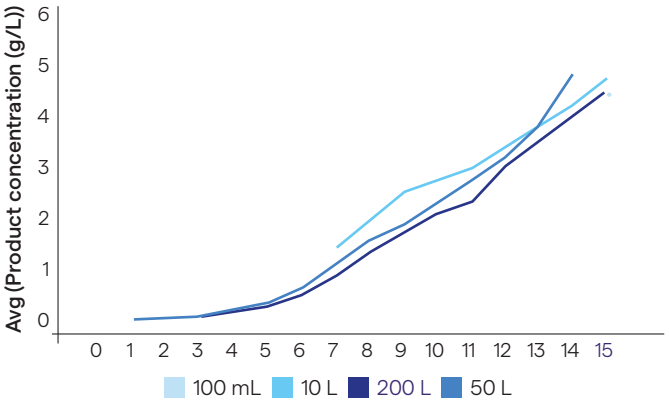


Figure 3.
Graph showing product concentration (mg/L) over a 15-day run using Lonza's TheraPRO® CHO Production Medium in cell culture vessels ranging from 100 mL to 200 L.

Lonza's TheraPRO® CHO Production Medium and Feed also support consistently high product yield, regardless of scale. 15-day fed-batch cell culture runs yielded between 4.5 and 5 g/L of monoclonal antibody (mAb) product across vessel sizes (Figure 4). Accordingly, the TheraPRO® CHO Production Medium and Feed can help support cost-effective large-scale protein manufacturing campaigns.

Product purity, isoform distribution, and glycosylation patterns remain unaltered

Culture Scale	Purification	Total aggregate measured by GP-HPLC (%)	Total monomer measured by GP-HPLC(%)
0.25 L	Small-scale Pr A resin tips	0.42	99.56
10 L	Small-scale Pr A resin tips	0.63	99.37
50 L	Small-scale Pr A resin tips	0.47	99.53
200 L	Small-scale Pr A resin tips	0.41	99.58

Table 1:
Gel-permeation chromatography (GPC) data showing low product aggregation across cultivation scales when using Lonza's TheraPRO® CHO Production Medium in fed-batch CHO cell culture.

In each of the different cell culture vessels we tested, the Lonza TheraPRO® CHO Production Medium and Feed delivered high monomer percentages (>99.3%), demonstrating its ability to support consistent production of pure, high-quality proteins across scales (Table 1).

Charge variant distribution across different scales

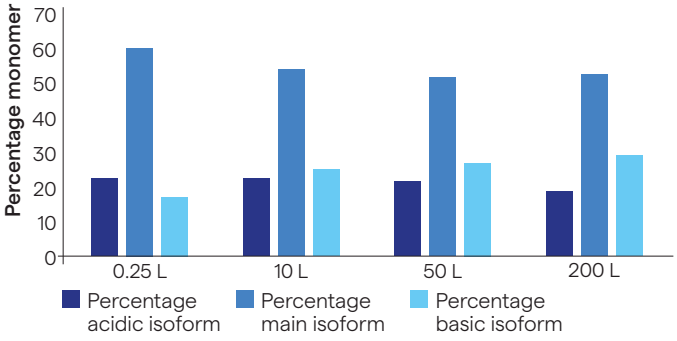


Figure 4.
Bar chart showing the percentage of acidic, main, and basic isoforms of antibodies purified from different scales of fed-batch cultivations using the Lonza TheraPRO® CHO Production Medium and Feed. Charge variant distributions were assessed using imaged capillary isoelectric focusing (icIEF).

Antibody charge variants have the potential to influence product stability and biological activity. As such, it is critical that bioprocess scale-up does not significantly change the product's charge variant distribution. Our experiments show that, when using the Lonza TheraPRO® CHO Production Medium and Feed, the cultivation scale does not impact the relative abundance of charge variants (Figure 5).

Glycan profiles across different scales

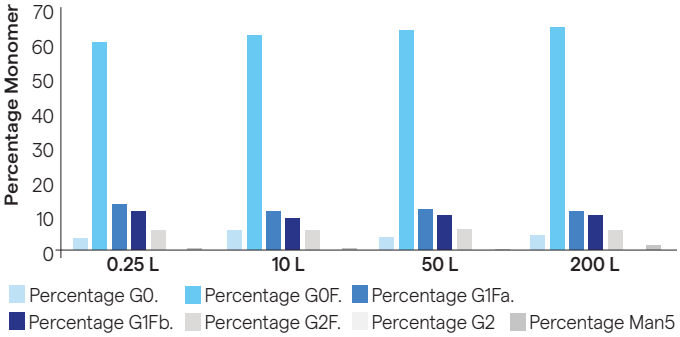


Figure 5.
Bar chart showing the most abundant antibody glycoforms purified from different scales of fed-batch cultivations using the Lonza TheraPRO® CHO Production Medium and Feed.

Every mAb product has a characteristic glycan profile. Vari-ation in this profile can significantly impact product activity, stability, and safety, and therefore it is imperative that the profile is not altered during bioprocess scale up. When we assessed the glycan profiles of the mAb produced in scalability analysis, we found that the TheraPRO® CHO Medium and Feed maintained a consistent glycan profile distribution (Figure 6).

Exceptional ease-of-use, reduced time and errors

In addition to offering the same performance across cultivation scales, the TheraPRO® CHO Production Medium and Feed are exceptionally easy to use, each comprising a single powder and requiring just 1 hour and 1.5 hours to prepare, respectively. As a result, companies can benefit from significant time savings (and thus lower operational costs), and reduced preparation complexity, helping to minimize the risk of operator error.

TheraPRO® CHO Production Medium and Feed customers also get access to Lonza's world-class technical support to ensure easy use and swift troubleshooting. Additionally, Lonza offers robust protocols for a range of CHO cell culture scales — protocols that have been proven to deliver exceptional cell culture performance and can help save time and minimize risk in process development and scale-up.

Ensure consistent performance and seamless scale-up with a robust CHO Media System

Scaling a CHO cell culture process from R&D to pilot and production scales is a highly complex task fraught with risk. The consequences of getting it wrong can be significant, both for drug developers and for patients in need of new and effective treatments.

To maximize chances of a seamless scale-up, companies must not only understand (and scale according to) the right scale-independent parameters, but also ensure that their cell culture medium and feed are robust to small environmental fluctuations, and can accommodate the elevated localized nutrient consumption commonly seen in larger scale bioreactors.

The TheraPRO® CHO Production Medium and Feed was designed with these needs in mind, offering an easy-to-use, robust solution for simplified, de-risked bioproduction scale-up. With it, customers can expect comparable viable cell concentration, viability, metabolic profiles, product concentration, and product quality, whatever their cultivation scale.

Lonza has the bioprocessing expertise, experience, and solutions to help support your scale-up success. To find out more about the TheraPRO® CHO Media System, visit our website, or reach out to one of our bioprocessing 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

References:

1. **Global Protein Therapeutics Market Size, forecast to 2033**. *Spherical Insights.* February 2024. Accessed May 20, 2024. https://www.spherica-linsights.com/reports/protein-therapeutics-market.
2. **Sieblist C, Jenzsch M, Pohlscheidt M.** *Equipment characterization to mitigate risks during transfers of Cell Culture Manufacturing Processes*. Cytotechnology. 2015;68(4):1381-1401. doi:10.1007/s10616-015-9899-0
3. **Lara AR, Galindo E, Ramírez OT, Palomares LA.** *Living with hetero-geneities in bioreactors: Understanding the effects of environmental gradients on cells.* Molecular Biotechnology. 2006;34(3):355-382. doi:10.1385/mb:34:3:355
4. **Reddy JV, Raudenbush K, Papoutsakis ET, Ierapetritou M.** *Cell-culture process optimization via model-based predictions of metabolism and protein glycosylation*. Biotechnology Advances. 2023;67:108179. doi:10.1016/j.biotechadv.2023.108179
5. **Anane E, Knudsen IM, Wilson GC.** *Scale-down cultivation in mam-malian cell bioreactors–the effect of bioreactor mixing time on the response of CHO cells to dissolved oxygen gradients.* Biochemical Engineering Journal. 2021;166:107870. doi:10.1016/j.bej.2020.107870
6. **Zakrzewski R, Lee K, Lye GJ.** *Development of a miniature bioreactor model to study the impact of ph and dot fluctuations on cho cell culture performance as a tool to understanding heterogeneity effects at large-scale.* Biotechnology Progress. 2022;38(4). doi:10.1002/btpr.326
7. **Gaugler L, Hofmann S, Schlüter M, Takors R.** *Mimicking Cho large-scale effects in the single multicompartment bioreactor: A new approach to access scale-up behavior.* Biotechnology and Bioengineering. 2024;121(4):1243-1255. doi:10.1002/bit.28647
8. **Karimi Alavijeh M, Baker I, Lee YY, Gras SL.** *Digitally enabled approach-es for the scale up of mammalian cell bioreactors.* Digital Chemical Engineering. 22022;4:100040. doi:10.1016/j.dche.2022.100040
9. Xu **S, Jiang R, Mueller R,** *et al. Probing lactate metabolism variations in large-scale bioreactors.* Biotechnology Progress. 2018;34(3):756-766. doi:10.1002/btpr.2620

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-SP002 07/25

bioscience.lonza.com
lonza.com/therapro