

From Shaker to Pilot/Production Bioreactor: How Scale Up Assist Using the BioFlo® 720 Bioreactor Control System Can Help Your Antibody Production Workflow.

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Abstract

To scale upstream bioprocesses, an effective scale-up strategy is required to ensure the reproducibility of both cell culture growth and batch yields at large working volumes, ideally with little or no additional optimization. The Constant Power approach is a successful method for matching bioreactor yields at different scales. This study details the use of this new Scale Up Assist feature of the BioFlo 720 Bioreactor Control System to scale CHO batch cultures from 3 L to 10 L BioBLU® Single-Use Vessels and from the 10 L to 50 L Single Use Bioreactor (SUB) bag. Using a built-in constant power software algorithm, the Scale Up Assist specified what agitation and gassing set

points to use for different vessel sizes.

We cultivated Chinese Hamster Ovary cells (CHO) using BioBLU Single-Use Vessels and a Thermo Scientific™ HyPerforma™ 50 L SUB. Our results demonstrated that the parameters provided by the new Scale Up Assist software resulted in similar cell growth and monoclonal antibody (mAb) production yields for all three vessel sizes. Adoption of the controller's Scale Up Assist function can decrease process development time while also helping to ensure consistent mAb yields across vessel sizes and bioreactor platforms.

Introduction

Bioprocess development is often done at small or bench scale to save production costs, optimize ideal conditions for cultures, and shorten time to market.

An effective strategy is required during scale-up to ensure matching cell growth in cultures and similar mAb or other protein production yields from small, bench, and pilot scale. However, sometimes these strategies to scale-up cell culture processes are not as straightforward as we would like. Many strategies have been established to streamline the scale-up of protein and mAb production. These strategies

include keeping a constant tip speed across bioreactors, matching oxygen volumetric mass transfer coefficients ($\kappa_L a$), and maintaining constant impeller power per unit volume (P/V). We have utilized the Constant Power approach, manually calculated, for scale-up in both fermentation [1] and cell culture [10] applications. This method involves calculating impeller power consumption per unit volume using Equation 1:

$$P/V = N_p \times \rho \times N^3 \times d$$

N_p is the dimensionless impeller power number or Newton number [2], ρ is the density of water (1000 kg/m^3), N is the impeller speed (rpm), d the impeller outer diameter (m), and V is the working volume of the culture (m^3). This strategy has proven to be an effective method to maintain similar bioreactor yields from bench to pilot scale.

Our goal was to test the constant P/V based on the Scale Up Assist feature to automatically match mAb production yields from our CHO culture using BioFlo 320 and BioFlo 720 bioreactor control systems. The BioFlo 720 is a new bioprocess controller that is compatible with Thermo Scientific's existing line of single-use bioreactors (SUBs). This controller introduces an innovative new feature termed Scale Up Assist, a built-in software algorithm that automatically calculates controller set-points, greatly simplifying the process of scaling-up bioreactor mAb production processes. We employed the new Scale Up Assist feature to successfully scale our CHO culture from 3 L to 10 L BioBLU vessels and from 10 L to 50 L SUB.

However, no success in bioreactor scale-up can be achieved without a healthy and robust inoculum. We used Eppendorf's New Brunswick™ S41i CO_2 Incubator Shaker to prepare the initial suspension CHO inoculum, and seeded our bioreactor with log phase cells at the highest quality level, $> 98.9\%$ viability.

Furthermore, no cost effective mAb production can be achieved without an economical way for medium preparation. We used a novel method consisting of a modified BioBLU 50c Single-Use Vessel, which yielded substantial savings in both costs and resources such as additional equipment needs.

Materials and Methods

In these experiments, we used the BioFlo 720 Scale Up Assist to calculate the process parameters needed to scale-up from 3 L to 10 L and from 10 L to 50 L. We cultured our CHO cells using the BioFlo 320 and BioFlo 720 bioreactor control systems (Appendix Table 1A and B).

Procedure / mAb Production Workflow

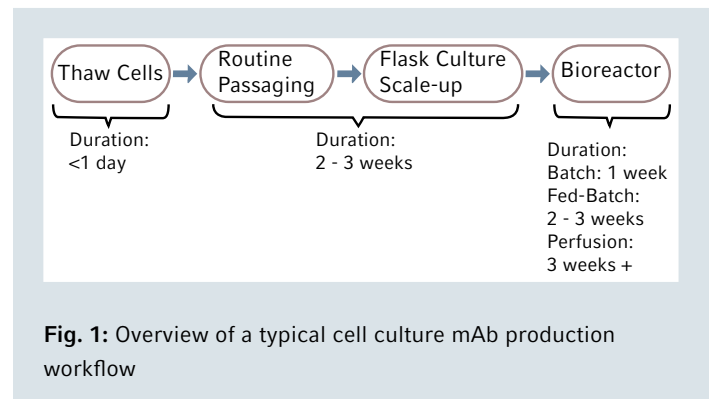
mAb Production workflow overview

Our workflow begins when we thaw our cells. This can typically take less than a day between flask and media preparations to introducing the cells into their new environment in the incubator (Figure 1).

We then move ahead to routine passaging and seed train or flask culture scale up. This step in our workflow can typically take anywhere from 2 to 3 weeks of culturing depending on the inoculation density we are targeting (Figure 1).

Lastly, we focus on our bioreactor step. The bioreactor phase of our workflow can vary depending on our bioprocess procedure. For example, our batch run typically lasts a week, our fed-batch runs can last 2 to 3 weeks and our perfusion can be three or more weeks long. For these experiments, we chose batch runs.

Now that our cell culture bioprocess workflow has been defined, we will break down each step further for each batch run in our experiments.



Thawing cells: Inoculum

For all experiments, we used a proprietary suspension CHO cell line producing a human monoclonal antibody (hmAb) from TPG Biologics, Inc., cultivated in Dynamis™ AGT™ Medium (Thermo Fisher Scientific®). The medium was supplemented with 8 mM L-glutamine, 1 % Antibiotic Antimycotic solution, and 1 % Gibco® Anti-Clumping Agent (Thermo Fisher Scientific) for a complete medium [3].

We prepared our cell culture inoculum by cultivating our cells in single-use, baffled bottom shake flasks (Corning®) with a 20 % maximum fill volume. We first thawed our cells from a previously cryopreserved vial and inoculated them into a 125 mL flask at a seeding density of 3×10^5 cells/mL. After our shake flask was seeded, we placed it into our New Brunswick S41i CO_2 Incubator Shaker. We set our shaking speed to 125 rpm and our CO_2 to 8 %.

Achieve the perfect inoculum!

A critical aspect to designing any cell culture bioprocess, especially regarding scale up, is to achieve matching growth



Fig. 2: New Brunswick S41i CO₂ Incubator Shaker

curves and production yields from shaker to pilot scale. Reducing shock factors such as temperature fluctuations can greatly reduce negative impact on your culture.

The New Brunswick S41i CO₂ Incubator Shaker combines precise temperature and CO₂ control with one of the best laboratory shakers available, resulting in high cell yields and viability (Figure 2). We have found the S41i incubator shaker to be an optimal tool for achieving the perfect inoculum.

An important feature of the S41i is the high temperature disinfection option. With a few simple clicks, your shaking incubator is clean and ready to go for your next bioprocessing challenge!

Routine passaging and seed train/flask culture scale-up workflow

After the initial thaw we allowed our cells to grow for a few passages before scale-up, in order to acclimate them to their environment. We determined that under our conditions the optimal passage schedule is every other day. After monitoring our cell growth and viability (determined to be >95 %), we increased our culture volume from 125 mL to 250 mL, and finally, to 1 L shake flasks. During this scale-up, flask inoculation density, percentage fill and other parameters remained the same. Using this culture method, each bioreactor in this experiment was inoculated with cells that were approximately at the same passage and duration of the culture post-thaw [3].

For each experiment, the number of flasks per scale-up step varied, based on the amount required for optimal target inoculation and working volume of each bioreactor. For all experiments, our target inoculation density was 5×10^5 cells/mL. We also prepared additional extra flasks in case of suboptimal performance. After enough cells were obtained, we took the amount of culture that was needed from our 1 L

flasks and combined them into a 2 L addition bottle (Eppendorf) that was prepared and autoclaved. We used this bottle method to inoculate our BioBLU 3c and 10c Single-Use Vessels.

For our 50 L SUB run, we needed a larger volume of inoculum. To reach this goal, we inoculated a BioBLU 10c Single-Use Vessel with a working volume of 10 L. Once growth in our bioreactor reached approximately 2×10^6 cells/mL, we then welded our harvest line on to our BioBLU 10c and inoculated our 50 L SUB (Figure 3).

Our scale-up seed train strategy used for the 40 L run can be applied to larger vessel sizes. By seeding a BioBLU 10c vessel and allowing culture density to reach approximately 2×10^6 cells/mL, we were able to inoculate our 50 L SUB, with a working volume of 40 L, at 0.5×10^6 cells/mL.

Using this approach, we could inoculate the 250 L and the 500 L vessels in a similar seed train process flow (Figure 4). For example, to inoculate a 500 L run, we would prepare our initial culture in our S41i shaker. We would then scale-up our flask culture to inoculate a BioBLU 10c vessel. We would allow our culture to grow for a few days to achieve the calculated density needed to inoculate a 50 L SUB. We could then incorporate our 50 L SUB culture into our seed train by allowing the culture to grow for an additional few days to reach a high enough density to inoculate our 500 L SUB run.

Bioreactor control and process parameters

Vessels

The BioBLU 3c and 10c Single-Use Vessels were operated by a BioFlo 320 bioprocess controller. Our BioFlo 320 was also used at the 10 L-scale to prepare the amount of inoculum needed for the 40 L culture. The BioFlo 320 controller was set up according to the manual [4].

A Thermo Scientific HyPerforma 50 L Outer Support Container was used for the 40 L CHO culture and was controlled by the BioFlo 720 bioprocess control system. A LAUDA® VC1200 water-cooled temperature control unit (TCU) was connected to the support container to provide heating to the jacket.

We used a Thermo Scientific HyPerforma 50 L Bioprocess Container (BPC) with a 5:1 turndown ratio and drilled hole sparger (DHS) for our 40 L CHO culture. Our internal BPC pressure was measured using a PendoTECH® Single Use Pressure Sensor™ and monitored by our BioFlo 720 bioprocess controller.

40 L vessel preparation and bag inflation

The Thermo Scientific BPC was inflated using the BioFlo 720's Auto Inflate feature. The auto inflation function automatically introduces a specified amount of air into the BPC until it is fully inflated. The auto inflate program also has a built-in safety feature that will automatically shut down all gases in the event of high pressure being detected in the bag. Our pressure sensors were connected before inserting the bag into the outer support container by using the aseptic pressure sensor connector (Thermo Scientific).

Media filling process

Sterile medium was pumped into the BioBLU 3c and 10c vessels directly through a top addition line by welding onto the addition line with a Terumo tube welder and using one of the pumps located on the BioFlo 320 control station.

Prepared medium was pumped into the 50 L BPC through a top addition line using the 520R2 pump head integrated on the BioFlo 720 cabinet. The medium for our 40 L run was sterilized by pumping it through Polycap TC filters after being prepared as described above. After 40 L was pumped into the BPC, we switched on the temperature loop to warm the medium at 37°C for a minimum of 12 hours prior to inoculation.

Process parameters for all runs

For all cultures, we measured dissolved oxygen (DO) by using Mettler Toledo® ISM® DO sensors. Our DO setpoint was set to 50 % for all experiments and was controlled at setpoint using 3 - gas auto mixing (Table 1). The P and I values selected for these experiments were optimized by using a dissolved oxygen simulation as previously described [8].

We used an analog pH sensor for all of our experiments. Our pH setpoint was set to 7.0 with a deadband of 0.1 and was controlled via a cascade to CO₂ (acid) and 0.45 M sodium bicarbonate (base). For our 3 L culture, we sterilized

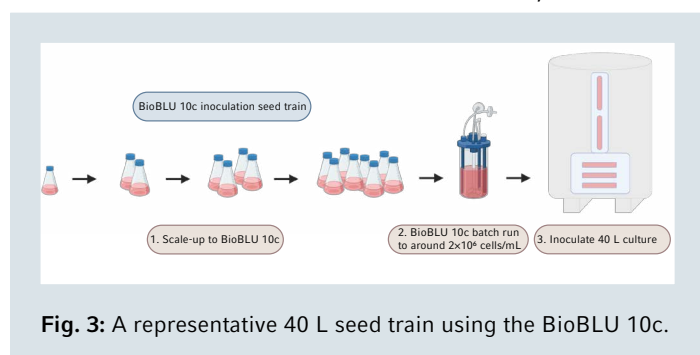


Fig. 3: A representative 40 L seed train using the BioBLU 10c.

our pH sensor after calibration in a sterilizing pouch and inserted the sensor aseptically into a spare Pg 13.5 port on the BioBLU 3c vessel in our Biosafety cabinet. Our pH sensor was calibrated based on the protocol in the BioFlo 320 manual [5]. For the 50 L culture, all sensors were inserted into probe assembly sleeves (Thermo Scientific) and autoclaved. The sensors were then inserted into the BPCs via the front sensor quick connectors, after inflation but prior to liquid filling. DO sensors were calibrated as previously

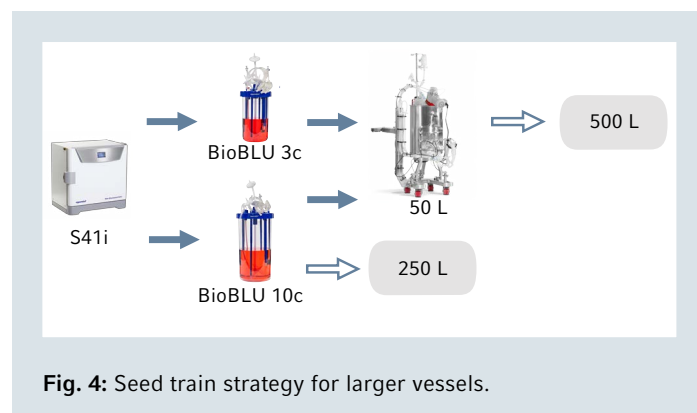


Fig. 4: Seed train strategy for larger vessels.

described [6].

Dissolved CO₂ was monitored using a Mettler ISM CO₂ sensor that was also aseptically inserted in the Biosafety cabinet into a spare port on the BioBLU vessels after being sterilized separately. For our 40 L run, the sensor was sterilized using the SUB probe assembly and aseptically inserted into the SUB.

For all experiments, temperature was controlled at 37°C and remained constant for the duration of the runs.

Scale Up Assist feature

The BioFlo 720 bioreactor control system was designed to save time and to mitigate risks. (Figure 5). The well-known BioFlo software has been enriched with a variety of new features to automate the workflow, saving time and simplifying repetitive tasks, such as the Scale Up Assist. Whether scale-up strategy is based on constant P/V or constant tip speed, the Scale Up Assist calculates all critical parameters needed, including gas flow rates and agitation with vessel specific data automatically populated in the software.

Critical parameters for our experiments, including agitation and gas flow rates, were calculated by the new Scale Up Assist function on the BioFlo 720 (Figure 6). We used a constant power/volume (P/V) ratio of 20 for our scale-

Table 1: Overview of process configurations and setpoints for all cell culture runs.

Parameters	3c Setpoints	10c Setpoints	50 L Setpoints
Controller	BioFlo 320	BioFlo 320	BioFlo 720
Working volume	3 L	10 L	40 L
Agitation	151 rpm	126 rpm	169 rpm
Temperature	37 °C		
DO Sensor	polarographic sensor		
DO Setpoint	50 %, (P= 2; I= 0.09)	50 %, (P= 2; I= 0.09)	50 %, (P= 2.5; I= 0.09)
pH Sensor	potentiometric sensor		
pH Setpoint	7.0 (deadband = 0.1), cascade to CO ₂ (acid), cascade to 0.45 M sodium bicarbonate (base)		
Target Inoculation Density	0.5 x 10 ⁶ cells/ mL		
Gas strategy	3 – gas auto mixing		
Gassing range	0.04 SLPM – 1 SLPM	0.04 SLPM – 1 SLPM	0.04 SLPM – 13.33 SLPM



Fig. 6: BioFlo® 720 Scale Up Assist Screen.

Sampling and analytics

We took two samples from each bioreactor daily, one in the morning and one in the evening, to check offline values such as cell density, viability, glucose, ammonia (NH₃), lactate, and hMAb concentration. To collect the highest quality sample from the growing culture in our BioBLU bioreactors, we connected a sterile 5 mL syringe to the sample port Luer Lock and removed a dead volume of 3 mL. We then collected a second 3 mL sample in a new syringe to provide a fresh, viable sample for analytics [3].

For our 40 L SUB run, we used sterile 50 mL Labtainer™ BioProcess Container bags as our sampling bags and welded them onto our sampling line using our Terumo tube welder. We first welded on a fresh bag, used to remove a dead volume of 20-30 mL from our sampling line. We then welded this bag off and welded on a new bag to our sampling line. We took roughly the same amount of sample for our cell count and analytics. Lastly, we welded on a final bag so that we could weld off the bag needed for our sampling. This is designated as the next sample time’s dead volume bag.

We measured cell density and viability (via the trypan blue exclusion method) using a Vi-Cell® XR Viability Analyzer (Beckman-Coulter®). We confirmed our pH values offline using an Orion Star™ 8211 pH-meter (Thermo Fisher Scientific). Using the offline pH value, we restandardized the pH calibration on the controller daily, to prevent any discrepancy between online and offline measurements. We measured glucose, ammonia, glutamate, lactate, and hMAb using a Cedex® Bio Analyzer (Roche Diagnostics®) [3].



Fig. 5: The new BioFlo 720 bioreactor control system with 250 L SUB.

up calculations. Using this P/V ensured all bioreactors could be operated at reasonable tip speeds for their vessel size and geometry. The power numbers used for these calculations were determined experimentally for our BioBLU vessels in the Eppendorf applications laboratory and were provided by Thermo Scientific for the 50 L BPC [7]. These values have been programmed into the BioFlo 720 and are automatically activated when the corresponding vessel is selected in the Scale Up Assist screen (Figure 6).

Results

Throughout our experiments, we monitored our metabolic profiles twice a day, to observe how our culture was growing and to intervene if we detected any changes in growth or the health of our cells. Our metabolic profile for our 40 L SUB run is shown in Figure 7 as an example.

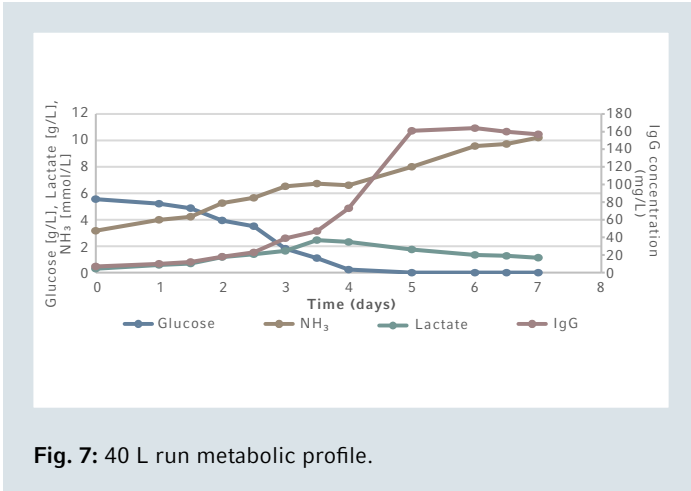


Fig. 7: 40 L run metabolic profile.

The actively growing cultures depleted the initially supplied glucose by day 5. Lactate remained under 2.5 g/L for the duration of the run. Ammonia concentrations rose every day to a toxic level of 10.2 mmol/L by day 6. We reached 164 mg/L of IgG by day 6 (Figure 7).

Cell growth comparisons

The cell growth curves for all three vessel sizes are shown in Figure 8. By using the parameters calculated by the BioFlo 720 Scale Up Assist feature, we were able to match the growth profiles across all platforms in multiple batch runs.

These results demonstrate that the new Scale Up Assist feature can decrease process development time while maintaining cell yields across vessel sizes and bioreactor platforms. Antibody production is shown for all runs in Figure 9.

All of our runs had similar results in IgG production when they reached completion.

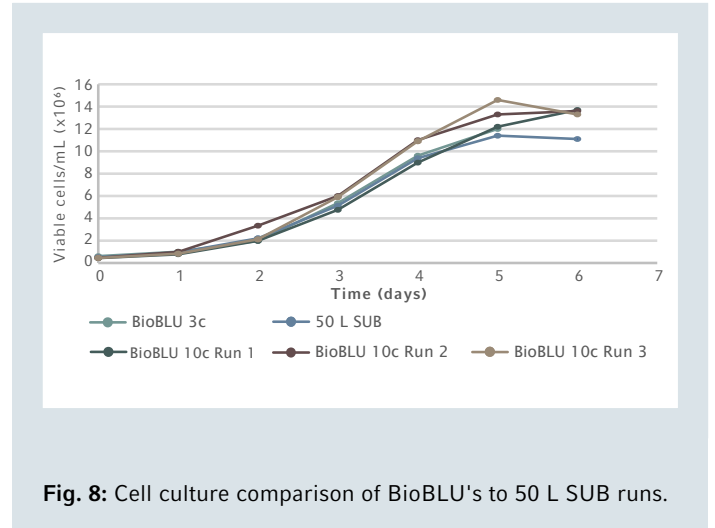


Fig. 8: Cell culture comparison of BioBLU's to 50 L SUB runs.

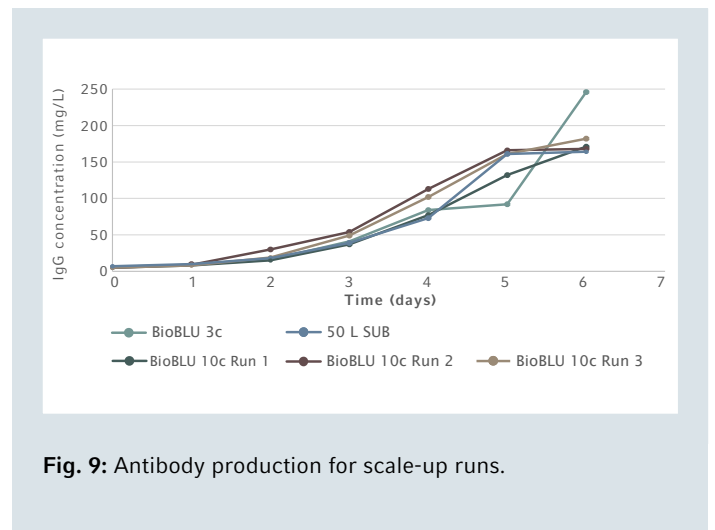


Fig. 9: Antibody production for scale-up runs.

Conclusion

There are several possible strategies for scaling up bioreactor cultures. Matching $\kappa_L a$ coefficients is one method that has been utilized with great success. This method requires extensive measurements of oxygen transfer under several different conditions. Gas flow, agitation, temperature, and bioreactor configuration can all impact $\kappa_L a$ constants and must be taken into account. Matching impeller tip speeds is an option that simplifies the scale-up process. However, this strategy may result in reduced mixing performance in larger vessels [9]. Maintaining constant power is another straightforward scale-up strategy. All that is required is a measurement of vessel power number to calculate Equation 1. Eppendorf and Thermo Scientific have measured the power numbers for a variety of their vessels, simplifying the use of the new Eppendorf Scale Up Assist feature to scale from their Eppendorf bench-scale single-use vessels to Thermo Scientific's pilot and production scale single-use vessels.

The BioFlo 720 Scale Up Assist application is convenient and straight forward to use. The Scale Up Assist feature allows operators to tailor their processes using constant power or tip speed. This allows users to pursue either strategy, or compare them to determine which one works best for their application. Users then select their vessels from the drop-down menus. Vessel characteristics, such as impeller diameter and power numbers, are automatically programmed. These steps are facilitated through a user-defined vessel option that can be manually adapted. The Scale Up Assist then automatically calculates the critical parameters, such as agitation and maximum gassing, to successfully scale their culture from one vessel to another.

In conclusion, we believe that these results demonstrate the effectiveness of the Constant Power strategy. Results that are shown using this strategy, indicate that it is possible to match yields from a 3L to a 40 L bioreactor culture.

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Appendix

App. Table 1A: Materials and equipment used for all cell culture runs in this study.

Material/Equipment	Supplier	Order No.
Cell Line		
Suspension CHO cell line with mAb	TPG Biologics, Inc	Proprietary
Cell Biology Workflow		
New Brunswick S41i CO ₂ Incubator Shaker	Eppendorf	S411120010
125 mL VWR® Erlenmeyer Flasks, Polycarbonate, baffled, Sterile	VWR International	89095-262
250 mL VWR Erlenmeyer Flasks, Polycarbonate, baffled, Sterile	VWR International	89095-270
1 L VWR Erlenmeyer Flasks, Polycarbonate, baffled, Sterile	VWR International	89095-286
Vi-CELL XR Cell Viability Analyzer	Beckman Coulter	731050
Cedex BIO analyzer	Roche Diagnostics	06395554001
ThawSTAR® CFT2 Instrument	MedCision®	MCS-601
Orion Star A211 pH meter	Thermo Fisher Scientific	STARA2116
Media Preparations		
BioFlo 120 Bioreactor Control System	Eppendorf	B120ACS008
BioBLU 50c Single-Use Vessel, Macrosparger	Eppendorf	M1363-0129
Dynamis AGT medium	Thermo Fisher Scientific	A2617504
Anti-Clumping Agent	Thermo Fisher Scientific	0010057DG
Antibiotic Antimycotic	Thermo Fisher Scientific	15240062
L- Glutamine	Thermo Fisher Scientific	25030164
Polycap TC 36 Capsule Filter, 0.2/0.2 µm, sterile	Cytiva	6714-3602
C-Flex®, Opaque White, 1/8" ID x 1/4" OD;	Cole Parmer®	EW-06424-67
C-Flex® Tubing, 1/4" ID 3/8" OD	Fisher Scientific	50-153-9381
Silicone Tubing 1/4" ID 3/8" OD	Eppendorf	M0740-2542
Silicone Tubing 3/16" ID, 5/16" OD	Eppendorf	M0740-2505
520U/R Pump	Watson-Marlow	050.7141.10A

App. Table 1B: Materials and equipment used for all cell culture runs in this study.

Material/Equipment	Supplier	Order No.
Bioreactor Workflow		
BioFlo 720 Bioreactor Control System	Eppendorf	1385200111
BioBLU 3c Single-Use Vessel, Macrosparger	Eppendorf	1386000300
BioBLU 10c Single-Use Vessel, Macrosparger	Eppendorf	1386141000
50 L Single-Use Outer Support Container	Thermo Fisher Scientific	SUB0050.9004
Thermo Fisher HyPerforma 50 L Bioprocess Container, 5:1 turn-down ratio, drilled hole sparger	Thermo Fisher Scientific	SH31073.01
Vi-CELL XR Cell Viability Analyzer	Beckman Coulter	731050
Cedex BIO analyzer	Roche Diagnostics	06395554001
Sterile Tube Fuser Dry	Cytiva	28999602
Terumo SCD® IIB Welder	Terumo BCT	3NCC986
DO Sensor (ISM), 220 mm	Mettler Toledo	P0720-6653
DO Sensor (ISM), 320 mm	Mettler Toledo	P0720-6654
pH Sensor, 225 mm	Mettler Toledo	P0720-5584
pH Sensor, 325mm	Mettler Toledo	P0720-5580
CO ₂ Sensor (ISM), 220 mm	Mettler Toledo	P0720-6664
520U/R Pump	Watson-Marlow	050.7141.10A
Sodium Bicarbonate	Fisher Scientific	S631-3
Antifoam C Emulsion	Millipore Sigma	A-8011
SUB Probe Assembly	Thermo Scientific	SH30720.02
Aseptic Connector to Pressure Sensor	Thermo Scientific	SH31134.01
PendoTECH® Single Use Pressure Sensor™, 1/4" Hose Barb	Cole Parmer	UX-19406-21
C-Flex, Opaque White, 1/8" ID x 1/4" OD;	Cole Parmer	EW-06424-67
C-Flex Tubing, 1/4" ID 3/8" OD	Fisher Scientific	50-153-9381
Silicone Tubing 1/4" ID 3/8" OD	Eppendorf	M0740-2542
Silicone Tubing 3/16" ID, 5/16" OD	Eppendorf	M0740-2505
Addition bottle kit, 2 L	Eppendorf	M1362-9902
Addition bottle kit, 5 L	Eppendorf	M1362-9903
Thermo Scientific Labtainer Bio-Process Container (BPC), 50mL with 2 Ports, Luer Lock, 2D BPC	Thermo Scientific	SH3065711

Ordering information

Description	Order no.
BioFlo® 720 Control System with 3 integrated Watson-Marlow® 314 peristaltic pumps	1385200011
BioFlo® 720 Control System with 3 integrated Watson-Marlow® 314 and 2 integrated Watson-Marlow® 520R2 peristaltic pumps	1385200111
Bioprocess Container (BPC), Thermo Scientific®	
50 L	0045890089
100 L	0045890090
250 L	0045890091
Outer Support Container (OSC), Thermo Scientific®	
50 L	0045890041
100 L	0045890044
250 L	0045890042
Temperature Control Units (TCU)	
TCU VC1200 (Recommended for 50L, 100L)	0045530001
TCU VC2000 (Recommended for 250L)	0045530002



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