Efficient, high-titer monoclonal antibody production in a fed-batch process using single-use stirred-tank and rocking bioreactor systems

This application note shows the feasibility of monoclonal antibody (MAb) production in fed-batch processes using the single-use Xcellerex™ XDR-200 stirred-tank and ReadyToProcess WAVE™ 25 rocking bioreactor systems. Cell expansion was performed in a 15 L rocking bioreactor culture to seed a 200 L stirred-tank bioreactor culture. To compare the performance of ReadyToProcess WAVE 25 with that of XDR-200, 7 L of the 200 L culture was transferred to a parallel ReadyToProcess WAVE 25 system to a total culture volume of 10 L. MAb yields from the parallel productions were 5.0 and 4.9 g/L for XDR-200 and ReadyToProcess WAVE 25, respectively. The metabolite profiles were very similar in both bioreactor cultures. Together, the ReadyToProcess WAVE 25 and Xcellerex XDR bioreactor systems provide a powerful solution for robust, scalable production of recombinant proteins in a single-use format. In addition, the ReadyToProcess WAVE 25 is a versatile tool that can be used for both seed-train applications and process development.

Introduction

The XDR-200 system is part of the Xcellerex bioreactor platform, with systems ranging from 10 L to 2000 L maximum cultivation volume. The platform offers the benefits of single-use technology and stirred-tank design in a modular, turnkey bioreactor format developed to meet the requirements in a GMP manufacturing environment.

The ReadyToProcess WAVE 25 rocking bioreactor system is designed for fast installation and convenient handling, and delivers a reliable and accurate performance in scales up to 25 L. The system is suitable for process development, in research applications, seed trains, and small-scale production.

This application note demonstrates the use of ReadyToProcess WAVE 25 as a seed-train bioreactor and compares the performance of XDR-200 and ReadyToProcess WAVE 25 systems in MAb production using a CHO DG44 cell line cultured in ActiCHO™ Media System. The experimental layout is shown in Figure 1.

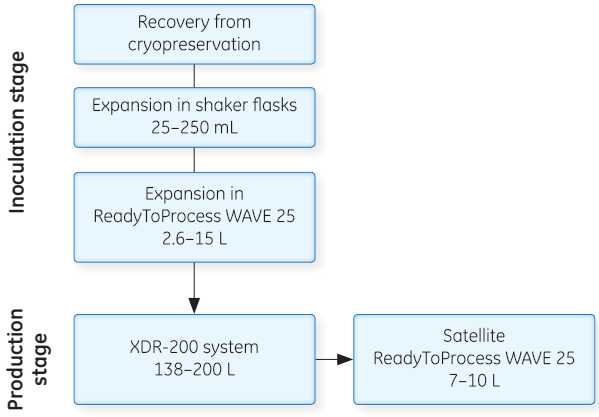

Fig 1. Cells were recovered from cryopreservation using standard methods. Initial cell expansion was performed in shaker flasks before further propagation in ReadyToProcess WAVE 25 to a final inoculum volume of 15 L. The inoculum was transferred to the XDR-200 system for MAb production. A portion of the XDR-200 culture was transferred to a ReadyToProcess WAVE 25 for parallel MAb production in a satellite bioreactor culture.

Materials and methods

Recovery of cryopreserved cells and inoculum expansion

CHO DG44 cells (licensed from Cellca GmbH, Laupheim, Germany) were recovered from cryopreservation according to a standard protocol and subcultured every third to fourth day. For inoculum expansion, ReadyToProcess WAVE 25 was used with operating parameters and conditions as listed in Table 1. The bioreactor system was operated in two steps: first at a low volume (2.6 L) and after three days at 15 L by addition of fresh medium.

Table 1. Operating parameters and conditions of the ReadyToProcess WAVE 25 inoculum bioreactor culture

Medium	ActiCHO P with 6 mM L-glutamine
Culture chamber	50 L Cellbag™ bioreactor
Rocking	
Low-volume stage	20 rpm, 6° rocking angle
Full-volume stage	20 rpm, 8° rocking angle
Gas flow	0.3 L/min
Seed cell concentration	0.3 × 10^6 viable cells/mL
pH set point	
Low-volume stage	No pH control (initial 7.5% CO_2 in headspace)
Full-volume stage	pH controlled at 7.0 with CO_2
Dissolved oxygen (DO) set point	40%
Working volume	2.6 L for initial 3 d in shaker flasks, 15 L to target cell concentration in ReadyToProcess WAVE 25
Target cell concentration at transfer	3.5 to 4.5 × 10^6 viable cells/mL
Viability criteria at transfer	> 95%

Production-stage cell cultures

The entire 15 L inoculum culture was aseptically transferred from ReadyToProcess WAVE 25 to the XDR-200 bioreactor system using a peristaltic pump (600 series pump, Watson-Marlow). After medium fill and inoculation to a total volume of 145 L, 7 L of the inoculated culture was transferred back to a ReadyToProcess WAVE 25 system, run as a satellite MAb production bioreactor. Operating parameters and conditions for the production bioreactors are listed in Table 2.

Table 2. Operating parameters and conditions of the production-stage bioreactors

	XDR-200	ReadyToProcess WAVE 25
Medium	ActiCHO P with 6 mM L-glutamine	ActiCHO P with 6 mM L-glutamine
Culture chamber	Standard bag assembly: Development	20 L Cellbag bioreactor with DO and pH sensors
Starting viable cell concentration	0.35 to 0.45 × 10^6 viable cells/mL	0.35 to 0.45 × 10^6 viable cells/mL
Inoculum volume	15 L	7.0 L transferred from XDR-200
Medium fill volume	130 L	N/A
Starting operating volume	145 L 138 L after transfer to satellite bioreactor	7.0 L
Impeller/rocker speed	150 rpm	20 to 30 rpm, 8° rocking angle, 30% rocking motion
pH set point	7.1	7.1
pH PID* settings	P: 0.5 I: 1.0 D: 0	Factory settings (automatically computed by the system)
Base for pH control	7.5% w/v $NaHCO_3$	7.5% w/v $NaHCO_3$
DO set point	40%	40%
DO PID* settings	P: 0.254 I: 4.083 D: 0	Factory settings (automatically computed by the system)
Air gassing	2 L/min head-space gassing DO-controlled gassing administered through 1 mm sparger Controller output: 0% to 20%: 1 to 5 L/min (sparged) 20% to 100%: 5 L/min (sparged)	On demand (from DO and pH control) 0.2 L/min total gas flow
Oxygen flow	On demand (from DO PID control) administered through 20 µm sparger Controller output: 0% to 20%: 0 L/min 20% to 100%: 0 to 5 L/min (sparged)	On demand (from DO PID control) max. 50% 0.2 L/min total gas flow
Antifoam	Medical antifoam C (Sigma-Aldrich) (added as required)	Medical antifoam C (Sigma-Aldrich) (added as required)
Feeds	ActiCHO Feed-A ActiCHO Feed-B 400 g/L D-glucose	ActiCHO Feed-A ActiCHO Feed-B 400 g/L D-glucose
Feed starting point	Day 2	Day 2
Daily feed volumes	ActiCHO Feed-A: 5.915 L ActiCHO Feed-B: 0.592 L Glucose: as required with target of 6 g/L excluding ActiCHO Feed-A	ActiCHO Feed-A: 0.295 L ActiCHO Feed-B: 0.0295 L Glucose: as required with target of 6 g/L excluding ActiCHO Feed-A
Harvest criteria	13 elapsed days and/or when culture viability was < 60%	13 elapsed days and/or when culture viability was < 60%

* Proportional-integral-derivative

Analytical methods

Analytical methods used are listed in Table 3.

Table 3. Analytical methods

Measured parameter	Assay type	Type of sample
Cell concentration and viability	Bioprofile FLEX™ analyzer (Nova Biomedical Corp.)	Cell suspension
Glucose	Bioprofile FLEX analyzer	Cell suspension
Lactate	Bioprofile FLEX analyzer	Cell suspension
Ammonium	Bioprofile FLEX analyzer	Cell suspension
Glutamine	Bioprofile FLEX analyzer	Cell suspension
Glutamate	Bioprofile FLEX analyzer	Cell suspension
Osmolality	Bioprofile FLEX analyzer	Cell suspension
Product concentration	CEDEX™ Bio analyzer (Roche)	Clarified cell culture supernatant

Results and discussion

Cell expansion for inoculum

After recovery and expansion of the CHO DG44 cell line in shaker flasks, the culture was transferred to a 50 L Cellbag bioreactor in ReadyToProcess WAVE 25. The bioreactor was inflated with a 7.5% CO_2 air mixture and inoculated at a cell density of 0.3 × 10^6 cells/mL in a total volume of 2.56 L. After approximately 72 h, the culture was expanded to 15 L by pump addition of production medium using ReadyToProcess Pump 25 and the ***Feed addition*** function of ReadyToProcess WAVE 25. Gassing and pH control were started at a set point of 7.0 (no base control). Cell growth and viability is shown in Figure 2. Online measurements of bioreactor weight, DO, and pH are shown in Figures 3 and 4. After another 48 h, the entire inoculum volume was transferred to the XDR-200 bioreactor at a viable cell concentration of 3.25 × 10^6 cells/mL.

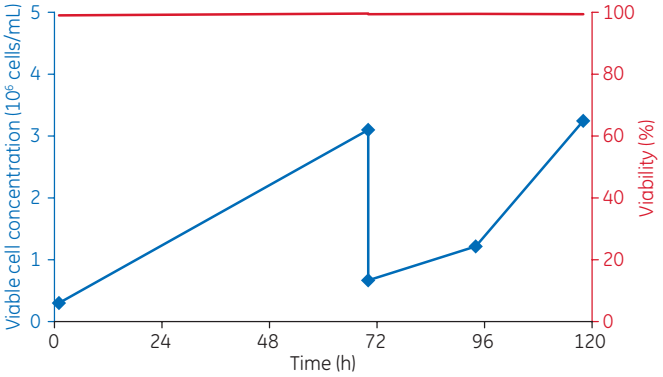

Fig 2. Cell growth and viability of the inoculum in the ReadyToProcess WAVE 25 system. The culture volume was expanded from 2.56 L to 15 L at 72 h.

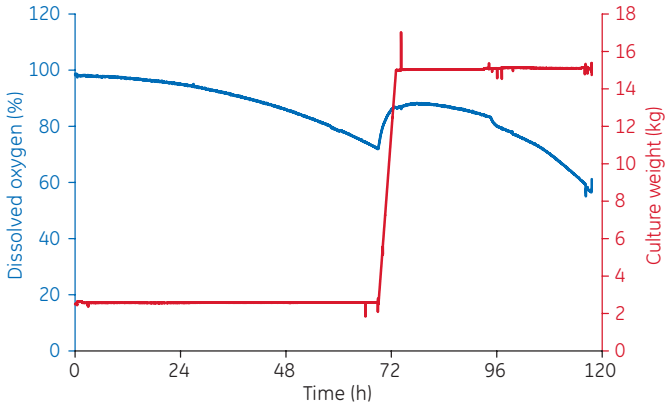

Fig 3. Online readings of ReadyToProcess WAVE 25 inoculum bioreactor volume and dissolved oxygen (DO).

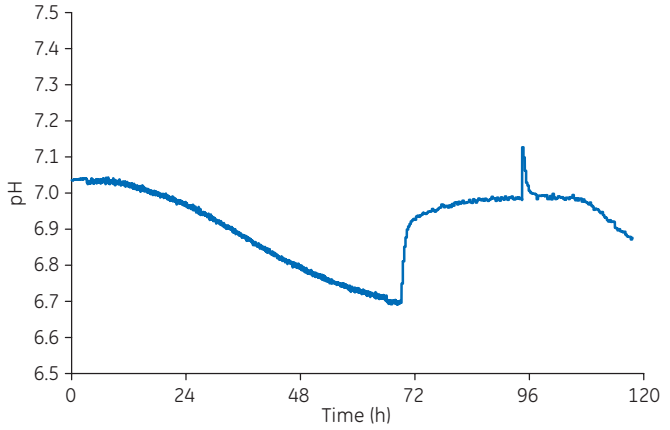

Fig 4. Online pH of the ReadyToProcess WAVE 25 inoculum bioreactor. An offline calibration adjustment was made at approximately 96 h, indicating that the actual pH before 96 h was approximately 0.1 pH units higher than the recorded pH.

XDR-200 bioreactor culture

One day prior to inoculation, the XDR-200 bioreactor was filled with 130 L production medium and was allowed to equilibrate to 37°C and pH 7.1. The bioreactor was inoculated with the entire volume of the ReadyToProcess WAVE 25 seed culture (15 L) to a final cell density of 0.38 × 10^6 cells/mL. The volume in the XDR-200 bioreactor after inoculation was 145 L, giving approximately a 10-fold volume expansion of the inoculum.

After equilibration, 7 L of the culture was transferred from the XDR-200 bioreactor culture into a satellite ReadyToProcess WAVE 25 bioreactor culture giving a XDR-200 culture volume of 138 L. The final volumes at harvest after daily feed-additions were calculated to be approximately 200 L for XDR-200 and 10 L for ReadyToProcess WAVE 25.

As shown in Table 4 and Figure 5, both cultures exhibited very similar growth profiles, with three distinct growth phases: an initial rapid growth phase for the first approximate 72 h; followed by a slower growth phase; and after approximately 144 h, a plateau and death phase with decreasing viability until harvest. The cultures were harvested with a viability of 65.3% to 73.0%. Both cultures exhibited similar productivity (Figure 6 and Table 4).

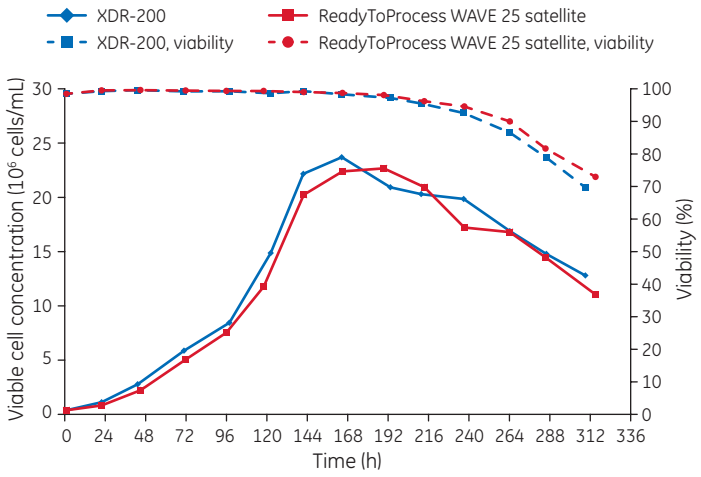

Fig 5. Viable cell concentration and viability of the parallel production cultures. Both cultures showed similar growth and viability profiles. Samples were taken just prior to feeding.

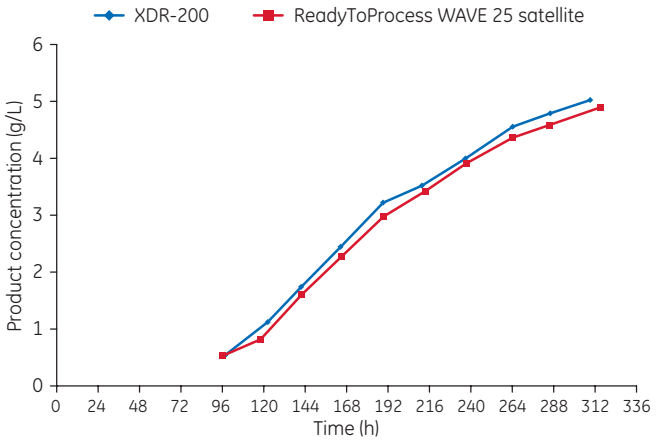

Fig 6. MAb concentration in the two parallel production cultures. Samples were taken just prior to feeding.

Both cultures exhibited an initial fast increase in lactate concentration during the exponential growth phase, followed by a reduction in the slower growth phase, and with constant low residual concentrations after the maximum cell concentration was reached and until harvest (Fig 7).

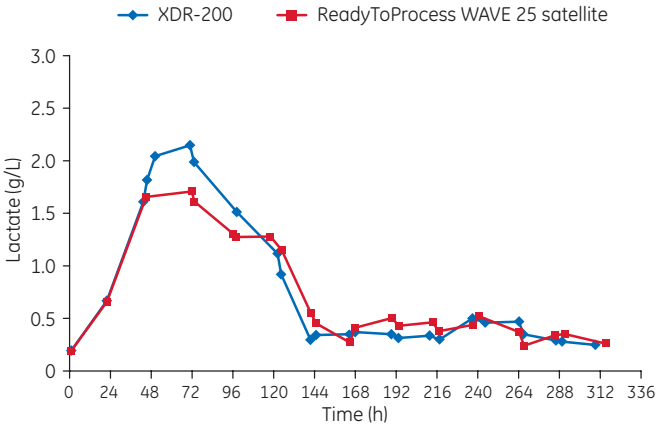

Fig 7. Lactate concentration in the two parallel production cultures. Samples were taken just prior to and after feeding.

Also the ammonium concentration profiles were similar for both cultures (Fig 8). After an initial rapid increase during the first approximate 48 h, concentrations steadily decreased until approximately 120 h of culturing, after which the ammonium concentrations increased again until time of harvest. The variability in ammonium production and consumption at different time points during the cultures can be associated with glutamine synthetase activity and with the varying metabolic rates of glutamine and other amino acids during the cell cycle. For example, there is a clear shift in metabolism around 72 h, where the lactate production rate slowed down and the glutamine production rate increased. This time point is when cells shift from fast growth rates, with a high level of glycolysis and high glutamine consumption, to lower growth rates and a higher energy production in the tricarboxylic acid (TCA) cycle. As glutamine is produced from glutamate and ammonium by the enzyme glutamine synthetase, these three compounds are closely linked, which is also reflected in Figures 9 and 10. Glutamate was supplied in the feeds, whereas glutamine was only available in the base medium.

Table 4. Results from the bioreactor MAb production cultures

Culture	Culture max. viable cell count (10^6 cells/mL)	Harvest viability (%)	Harvest product concentration (g/L)	Cell-specific product accumulation (pg/cell/d)
XDR-200	23.7	65.3	5.0	27
ReadyToProcess WAVE 25 satellite	22.7	73.0	4.9	28

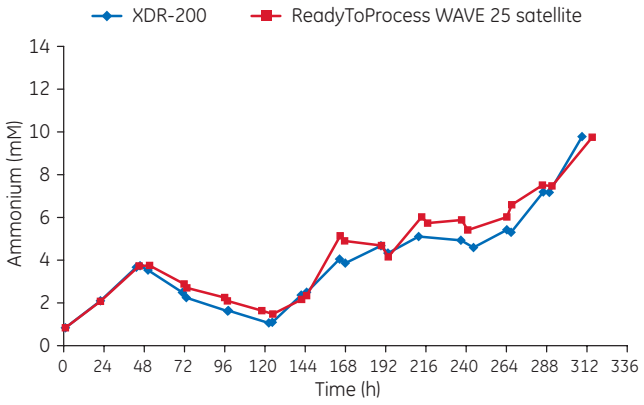

Fig 8. Ammonium concentration in the two parallel production cultures. Samples were taken just prior to and after feeding.

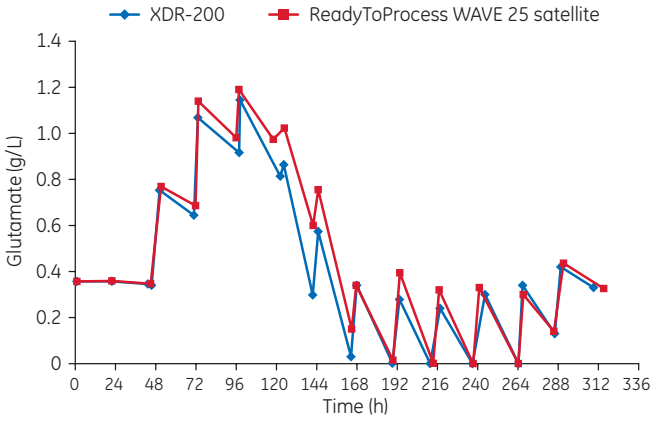

Fig 9. Glutamate concentration in the two parallel production cultures. Samples were taken just prior to and after feeding.

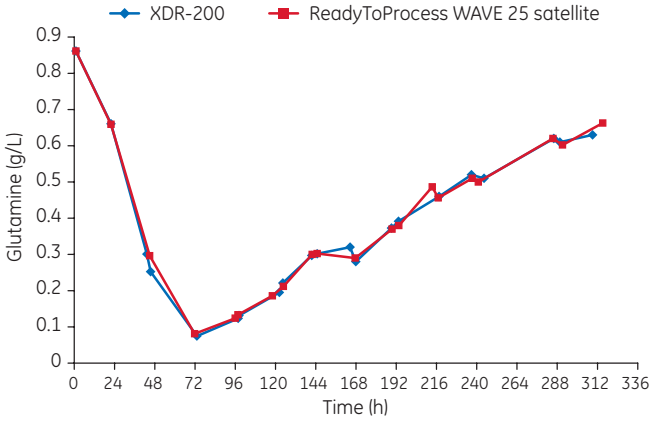

Fig 10. Glutamine concentration in the two parallel production cultures.

Osmolality was measured both before and after each feed addition. Mainly due to an increase in amino acid concentration, osmolality was increased by each feed addition (Fig 11). As amino acids were consumed, osmolality also dropped. However, as salts were also supplied in the feed and base additions, osmolality gradually increased during culture. In addition, unconsumed nutrients also contribute to the increase in osmolality.

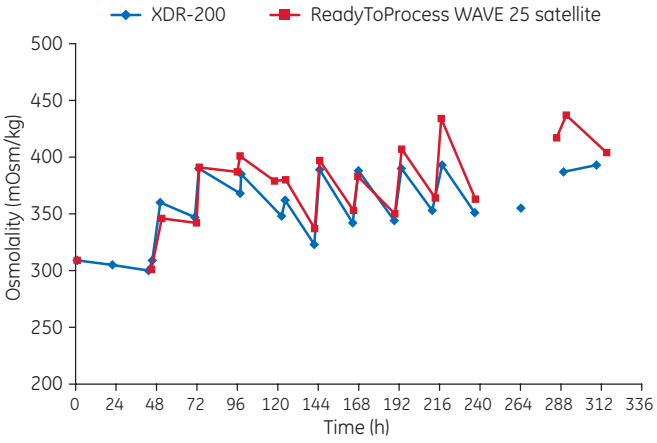

Fig 11. Osmolality in the two parallel production cultures. Samples were taken just prior to and after feeding.

The partial carbon dioxide pressure (pCO_2) profiles of the cultures are shown in Figure 12. As lactate is produced in the early stage of the culture, pCO_2 decreased to maintain pH and oppositely increases as lactate concentrations are lowered and basic components in the feeds and base control is added.

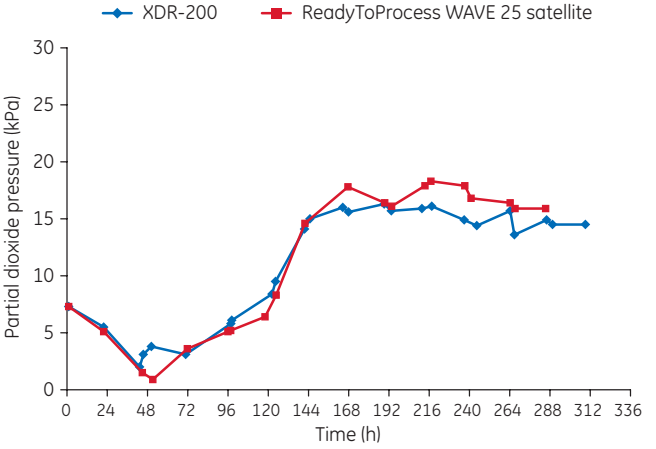

Fig 12. Partial carbon dioxide pressure in the two parallel production cultures.

Conclusion

The results show that the ReadyToProcess WAVE 25 can be used for both seed culturing and process development purposes. The culture results were shown to be comparable between ReadyToProcess WAVE 25 and XDR-200. Both bioreactor cultures exhibited similar cell growth and viability, metabolite content, and MAb productivity, with yields of 5.0 and 4.9 g/L for XDR-200 and ReadyToProcess WAVE 25, respectively. Although having a different vessel geometry, ReadyToProcess WAVE 25 gave a representative reflection of the process at larger scale using the XDR-200 system. In conclusion, the combination of ReadyToProcess WAVE 25 and Xcellerex bioreactor systems offers a reliable, scalable solution for recombinant protein production in scales up to 2000 L.

Ordering information

Product	Code number
ReadyToProcess WAVE 25, rocker	28-9880-00
ReadyToProcess™ CBCU Full	29-0440-81
ReadyToProcess Pump 25	29-0320-03
Tray 50	29-0444-74
Lid 50	29-0444-77
UNICORN™ 6.3.2 WrkStn-pure-BP	29-0469-18
Cellbag 20 L	29-0152-14
Cellbag 50 L	29-0152-16
Standard bag assembly: Development	29-0410-66
ActiCHO Level 1 CD Pwd Kit	29-0925-41

Related literature	Code number
ReadyToProcess WAVE 25, data file	29-0566-95
Disposable Cellbag bioreactors for WAVE Bioreactor™ systems, data file	28-9511-36
Xcellerex XDR cell culture bioreactor systems, data file	29-0929-25
UNICORN 6 control software, data file	28-9573-46

For more information on the XDR-200 bioreactor system, please contact your local sales representative.

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com/bioprocess

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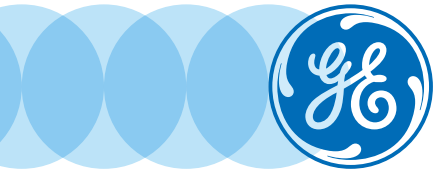

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