Scale-up of CHO cell fed-batch cultures in HyClone ActiPro medium supplemented with Cell Boost 7a and 7b

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CY13815-25May20-AN

Scale-up of CHO cell fed-batch cultures in HyClone™ ActiPro™ medium supplemented with Cell Boost™ 7a and 7b

This application note compares cell growth and recombinant protein production of multiple Chinese hamster ovary (CHO) cell clones when cultured in HyClone ActiPro basal medium and Cell Boost 7a and 7b feeding supplements. Results from shake flask cultures show high viable cell densities and protein production. In addition, scalability of the culture process was demonstrated in 2 and 50 L bioreactor cultures. Cell density and protein production were shown to be comparable between scales. Protein yields reached as high as 5 g/L in shake flasks, and as high as 5.5 g/L in bioreactor cultures.

Introduction

The most widely used cell lines for biopharmaceutical production of therapeutic proteins originate from CHO cells. These cells are robust in culture and are able to produce a variety of recombinant glycoproteins at high levels in large scales. ActiPro medium and Cell Boost 7a and 7b supplements are designed and optimized for large-scale protein production in fed-batch suspension culture using recombinant CHO cells. Chemically defined with no peptides or hydrolysates, ActiPro medium and Cell Boost supplements are animal-derived component-free (ADCF) and manufactured using current good manufacturing practices (cGMP). As ActiPro medium and Cell Boost 7a and 7b supplements do not contain hypoxanthine or thymidine (HT), their formulations supports the dihydrofolate reductase (DHFR) gene amplification and selection system. ActiPro medium and Cell Boost 7a and 7b supplements can be used together in a fed-batch process or the medium and supplements can be used in combination with other products to increase productivity of an existing process.

This application note demonstrates growth and productivity of CHO cells cultured in ActiPro medium and Cell Boost 7a and 7b supplements. Studies were performed with multiple CHO cell clones in 30 mL shake flasks cultures. To demonstrate scalability of the process, cultures were transferred to 2 L and 50 L bioreactor cultures and cell growth and productivity of selected clones were compared between scales.

Materials and methods

Studies in shake flask cultures

Three different proprietary CHO cell clones CHO-S (mAb producer), DG44 (mAb producer), and CHO-S (parental line, non-producer) were recovered from cryopreservation according to standard protocol and subcultured every third or fourth day. Once cells had recovered, they were inoculated in ActiPro medium. A minimum of three adaptation passages were completed until the cells adapted. Cells were considered adapted when their doubling time was less than 24 h. Following adaptation, cells were seeded into 30 mL volume in shaker flask at a density of 0.5×10^6 viable cells/mL. Starting on day three, each culture was fed Cell Boost 7a at 3% of vessel volume and Cell Boost 7b at 0.3% of vessel volume. Cultures were maintained at a minimum of 3 g/L of glucose by supplementing with a 250 g/L glucose concentrate as determined using Accutrend™ Plus glucose monitor (Boehringer Mannheim GmbH). All studies were performed in duplicate.

2 L bioreactor cultures

CHO-S (mAb producer) and DG44 (mAb producer) cell clones were expanded in 2 L bioreactor (Applikon Biotechnology) cultures. The bioreactor was filled with 2 L of ActiPro medium and equilibrated to 37°C and pH 7.0 before being seeded with 0.5×10^6 cells/mL. Starting on day three, each culture was fed Cell Boost 7a at 3% of vessel volume and Cell Boost 7b at 0.3% of vessel volume. Glucose was maintained at 3 g/L as measured with BioProfile™ FLEX analyzer (Nova Biomedical).

The cultures were run in chemostat mode: each day prior to feeding, the culture volume was drained to 2 L total volume. Antifoam (Sigma-Aldrich) was added as needed to minimize foaming. Table 1 lists the operating parameters and culture conditions.

Table 1. Operating parameters and conditions for 2 L bioreactor cultures

Medium	ActiPro with 6 mM L-glutamine
Supplements	Cell Boost 7a (3% of working volume) Cell Boost 7b (0.3% of working volume)
Culture chamber	2.5 L bioreactor
Mixing	350 rpm
Gas flow	Set by controller to maintain 50% dissolved oxygen (DO)
Seed cell concentration	0.5×10^6 viable cells/mL
pH set point	7.0
DO set point	50%
Antifoam	Antifoam C emulsion as needed
Working volume	2 L chemostat operation
Harvest criteria	Culture viability < 80%

50 L bioreactor cultures

Due to its high level of protein production, the DG44 (mAb producer) cell clone was chosen for expansion in 50 L Xcellerex™ XDR-50 bioreactor cultures. Cells were seeded at 0.5×10^6 cells/mL into a starting volume of 25 L of ActiPro medium. Starting on day three, the culture was fed Cell Boost 7a at 3% of the current volume, Cell Boost 7b at 0.3% of current volume, as well as glucose to maintain level at 5 g/L. Antifoam C was added as needed to prevent foaming. Cultures were fed to a final working volume of 50 L. Table 2 lists the operating parameters and culture conditions.

Table 2. Operating parameters and conditions for 50 L bioreactor cultures

Medium	ActiPro with 6 mM L-glutamine
Supplements	Cell Boost 7a (3% of working volume) Cell Boost 7b (0.3% of working volume)
Culture chamber	50 L bioreactor
Mixing	95 rpm
Gas flow	Set by controller to maintain 50% dissolved oxygen (DO)
Seed cell concentration	0.5×10^6 viable cells/mL
pH set point	7.0
DO set point	50%
Antifoam	Antifoam C emulsion as needed
Working volume	Fed-batch operation Start: 25 L Final: 50 L
Harvest criteria	Culture viability < 80%

Analytical methods

Analytical methods used are listed in Table 3.

Table 3. Analytical methods

Measured parameter	Assay	Sample
Cell concentration and viability	Vi-CELL™ XR cell viability analyzer (Beckman Coulter)	Cell suspension
Glucose	Bioprofile Flex analyzer Accutrend Plus glucose monitor	Cell suspension
Lactate	Bioprofile Flex analyzer	Cell suspension
Glutamine	Bioprofile Flex analyzer	Cell suspension
Glutamate	Bioprofile Flex analyzer	Cell suspension
Product concentration	Octet™ QK384 (Pall ForteBio) ELISA	Clarified cell culture supernatant

Results and discussion

Studies in shake flask cultures

Before all cell clones were set up for terminal growth curves in fed-batch mode, the cells were adapted in culture medium (Fig 1). Viable cell densities were measured daily, and samples for productivity determination were taken. Two of the used clones produce mAbs and one is a parental line and does not produce recombinant protein. Results are shown in Figures 2 to 4.

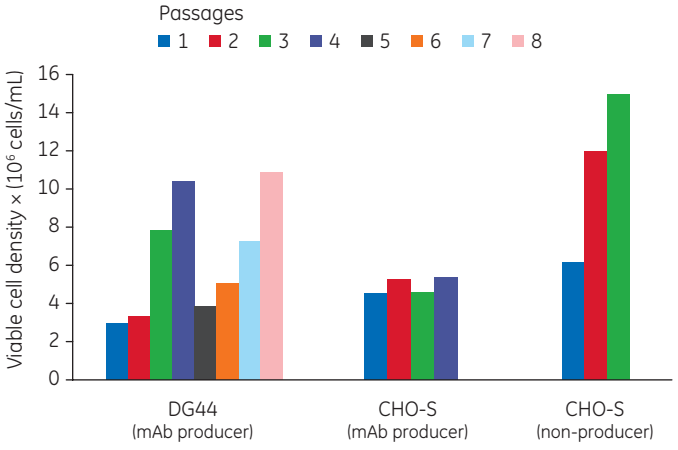

Fig 1. Comparison of DG44 (mAb producer) cells (adapted after eight passages), CHO-S (mAb producer) cells (adapted after four passages), and CHO-S (non-producer) cells (adapted after three passages) grown in ActiPro medium.

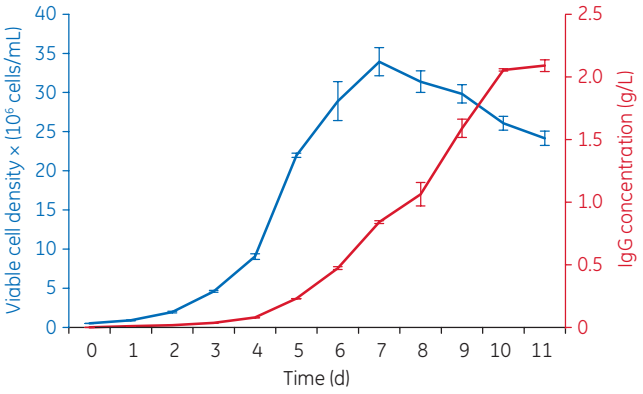

Fig 2. Growth and productivity of CHO-S (mAb producer) cells cultured in fed-batch mode. Viable cell density reached more than 30 × 10^6 cells/mL and IgG production reached 2.2 g/L.

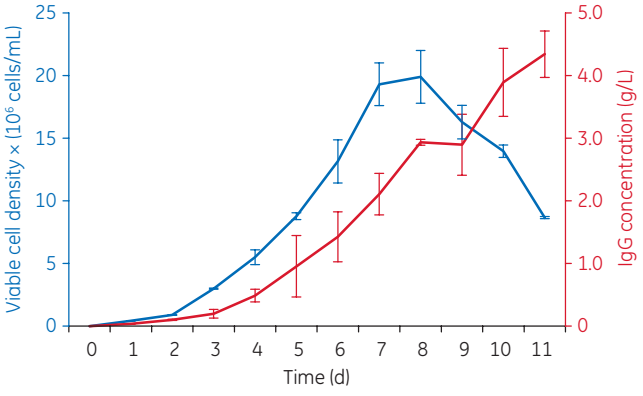

Fig 3. Growth and productivity of DG44 (mAb producer) cells cultured in fed-batch mode. Viable cell density reached 20 × 10^6 cells/mL by day 8 and IgG production reached 4.3 g/L.

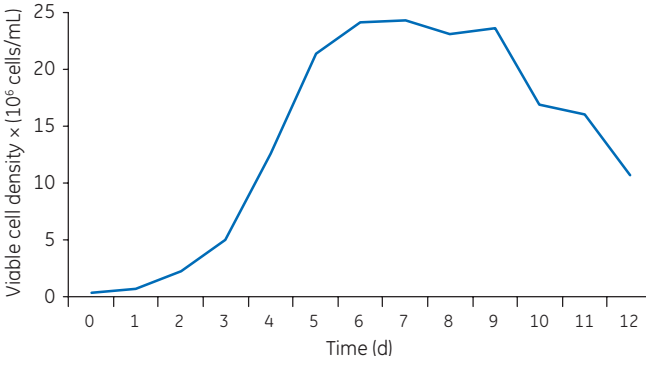

Fig 4. Growth of CHO-S cells cultured in fed-batch mode. Viable cell density reached more than 24 × 10^6 cells/mL by day 7. No productivity data is shown, as the CHO-S clone used here is a parental clone and does not produce recombinant protein.

Bioreactor cultures

Scalability of selected CHO cell clones was demonstrated in 2 L, and 50 L bioreactor cultures for the DG44 (mAb producer) cell clone.

For the 2 L bioreactor cultures, both cell clones reached a viable cell density of greater than 20 × 10^6 cells/mL by day 6 (Fig 5A). The DG44 (mAb producer) cell clone is a higher IgG producer than the CHO-S (mAb producer) cell clone; however, both reached production levels as high as expected for these clones and protein production continued for the length of the runs (Fig 5B).

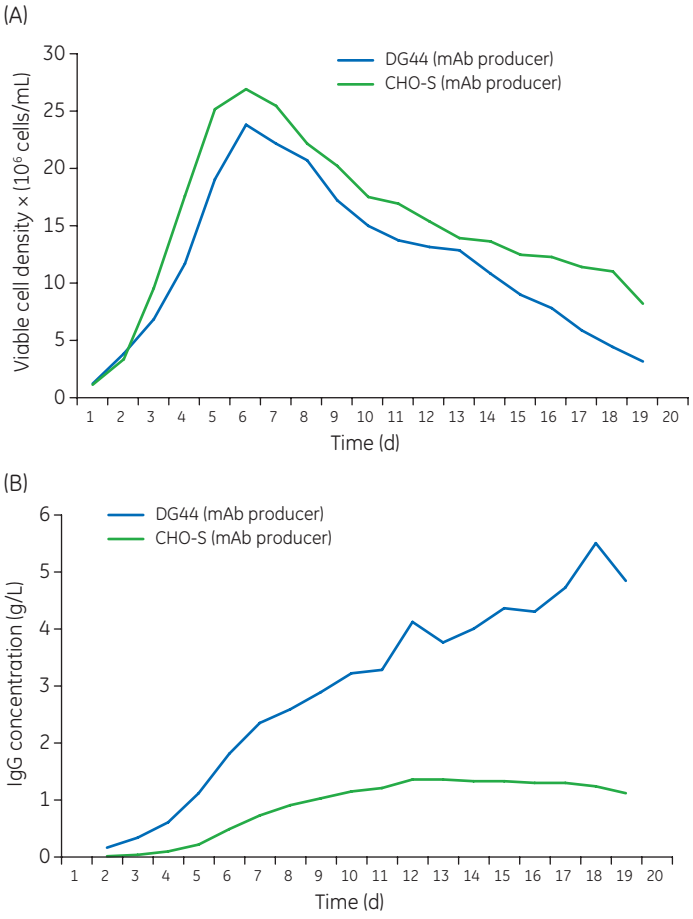

Fig 5. (A) Viable cell density and (B) productivity of DG44 (mAb producer) and CHO-S (mAb producer) cells in fed-batch bioreactor cultures.

For the 50 L bioreactor culture, cell density reached nearly 25 × 10^6 cells/mL by day 7 and productivity reached 5 g IgG /L by day 10 (Fig 6). Metabolite levels for the 50 L bioreactor culture are shown in Figure 7. As shown, the daily increase in glucose consumption correlates well with the cell density in the log phase of growth. Glucose was supplemented to maintain levels required for cell growth.

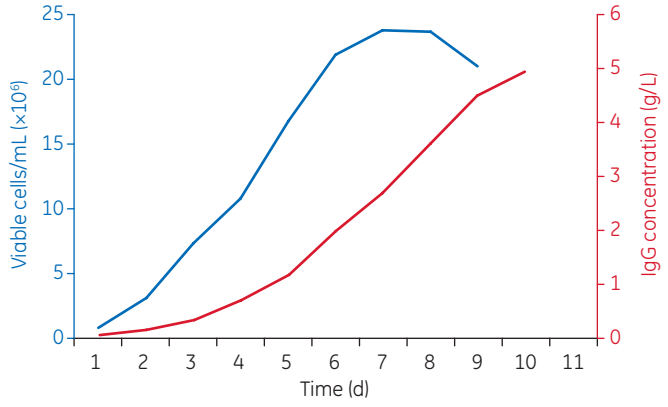

Fig 6. DG44 (mAb producer) cell growth and productivity in 50 L ActiPro bioreactor cultures.

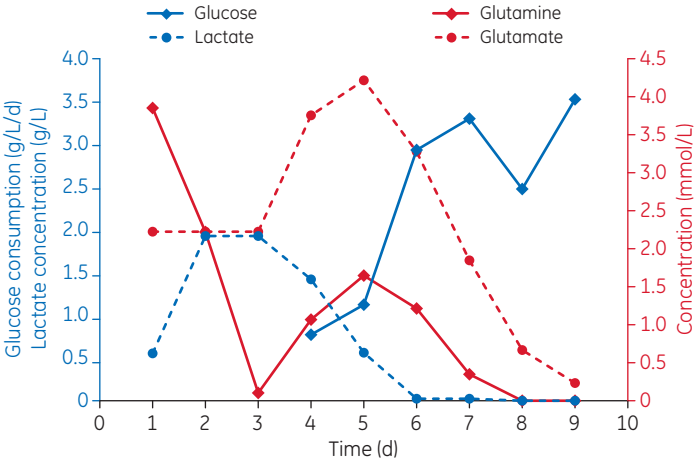

Fig 7. Metabolite concentrations for the 50 L bioreactor culture.

Conclusion

This work illustrates growth and productivity of multiple CHO cell clones cultured in ActiPro medium supplemented with Cell Boost 7a and 7b with regard to viable cell density and protein production in shake flask cultures. When transferring the cultures from shake flask to 2 and 50 L bioreactor cultures, selected clones showed similar performance to the shaker flask cultures.

Together, ActiPro medium and Cell Boost 7a and 7b supplements are shown to be a versatile cell culture platform that can be used with multiple CHO cell clones in both small- and larger-scale applications.

Ordering information

Product	Product code
HyClone ActiPro medium	SH31037
HyClone Cell Boost 7a	SH31026
HyClone Cell Boost 7b	SH31027

gelifesciences.com/hyclone

GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
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29175257 AB 01/2017

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