ActiPro medium and Cell Boost supplements: benchmarking, scalability, and protein production in CHO cell culture

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ActiPro™ medium and Cell Boost™ supplements: benchmarking, scalability, and protein production in CHO cell culture

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Abstract

The purpose of this work was to demonstrate the performance of HyClone™ ActiPro medium and Cell Boost supplements with multiple Chinese hamster ovary (CHO) cell clones with regard to both viable cell density and protein production when compared with other commercially available CHO cell media and supplements in shake flask cultures. Furthermore, we demonstrate scalability of ActiPro medium and supplements up to 50 L bioreactor scale, and determine the ability to utilize standard feed additions for multiple CHO clones.

Introduction

The most widely used cell lines in the bioprocess industry originate from CHO cells. With the large number of CHO clones in use, it is important to have a cell culture medium that supports high viable cell counts and productivity with multiple cell clones. A precise balance of nutrients is crucial for the optimal performance of these cell clones. ActiPro cell culture medium and feed supplements has a tailored formulation that supports viable cell counts of more than 25 million cells per mL and protein production greater than 5 grams per liter. High viable cell density and productivity across multiple CHO cell clones, from shake flasks to 50 L bioreactor cultures, demonstrate the versatility and scalability of the ActiPro medium and Cell Boost supplements.

Materials and methods

Shake flask cultures

The proprietary CHO clones CHO-S (MAb producer), DG44 (MAb producer), and DG44 (recombinant protein producer) were recovered from cryopreservation according to standard protocol and subcultured every third or fourth day. Once cells had recovered, they were inoculated into ActiPro or other commercially available media (Table 1). Note: for DG44 (MAb Producer) condition C was not available. A minimum of three adaptation passages were completed for each medium type. Following adaptation, cells were seeded in shaker flasks for fed-batch culturing in 30 mL medium, each supplemented according to medium manufacturer directions. Fed-batch studies were completed with each medium in duplicate. Seeding density was 0.5×10^6 viable cells/mL. Error bars on the results graphs represent ± 1 standard deviation.

Table 1. Culture media and feeds

Condition	Medium
A	ActiPro + Cell Boost 7a and 7b
B	Dynamis™ + EfficientFeed™ C + AGT™ (Thermo Fisher Scientific)
C	EX-CELL™ Advanced™ CHO + EX-CELL Advanced CHO Feed 1 (Sigma-Aldrich)
D	CD FortiCHO™ + EfficientFeed C + AGT (Thermo Fisher Scientific)
E	CD OptiCHO™ + EfficientFeed A (Thermo Fisher Scientific)
F	BalanCD™ CHO Growth A + BalanCD CHO Feed 1 (Irvine Scientific)

2 L bioreactor cultures

The CHO-S (MAb producer), DG44 (MAb producer), and DG44 (recombinant protein producer) cell clones were expanded in 2 L bioreactor cultures (Applikon Biotechnology). Cells were seeded at 0.5×10^6 cells/mL into a starting volume of 2 L of ActiPro medium. Starting on day three, each culture was fed Cell Boost 7a at 3% of vessel volume, Cell Boost 7b at 0.3% of vessel volume, and a glucose solution to maintain 3 g/L glucose as measured using BioProfile Flex™ analyzer (Nova Biomedical). These runs were maintained at a total volume of 2 L using a chemostat method: each day prior to feeding, the fluid levels were drained to a 2 L volume. Antifoam C (Sigma-Aldrich) was added as needed to minimize foaming.

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™ 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.

Results

In shake flask cultures, all ActiPro cultures consistently reached viable cell densities above 20×10^6 cells/mL across all culture methods tested (Fig 1–3). There was a strong correlation between cell concentration and protein production. Cells grown in ActiPro medium and Cell Boost supplements produced higher protein titers than when grown in other studied media (Fig 4–6). Two of the clones used in the comparison produce MAb and one produce a proprietary recombinant protein, indicating that ActiPro medium and supplements are capable of supporting high protein production in various protein production systems. The CHO cell clones showed similar growth and productivity profiles in all ActiPro cultures.

Scalability of selected CHO cell clones was demonstrated in 2 L and in 50 L bioreactor cultures for the DG44 (MAb producer) cell clone. All clones showed comparable viable cell density and protein production between culture scales (Fig 7–10). Optimization of the standard addition of 3% Cell Boost 7a and 0.3% Cell Boost 7b to each clone for adequate nutrition to support rapid growth during the log phase of cell growth could further enhance growth and production.

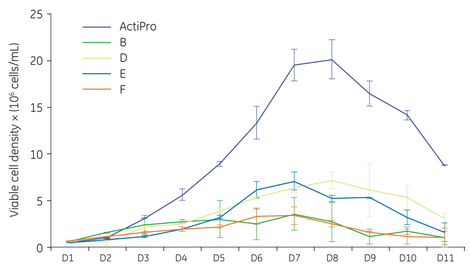

Fig 1. DG44 (MAb producer) cell growth.

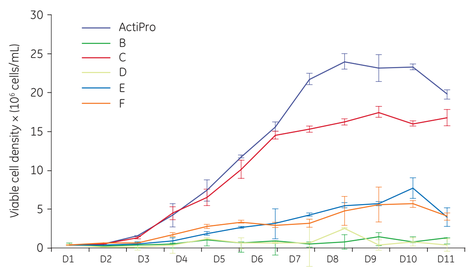

Fig 2. DG44 (recombinant protein producer) cell growth.

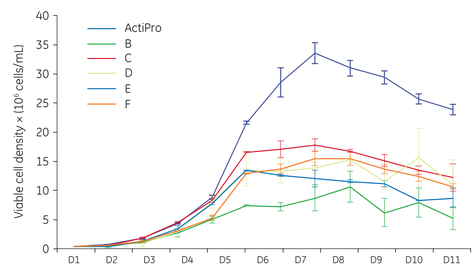

Fig 3. CHO-S (MAb producer) cell growth.

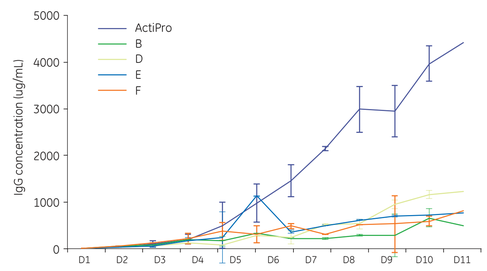

Fig 4. DG44 (MAb producer) productivity.

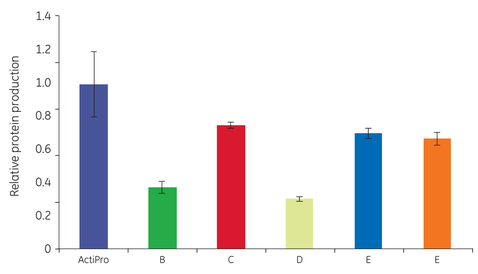

Fig 5. DG44 (recombinant protein producer) productivity (day 10).

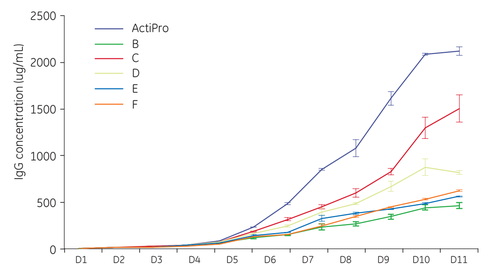

Fig 6. CHO-S (MAb producer) productivity.

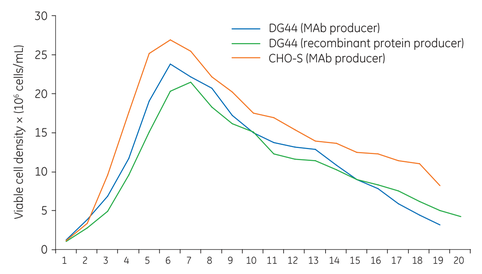

Fig 7. Cell growth in 2 L ActiPro fed-batch bioreactor cultures.

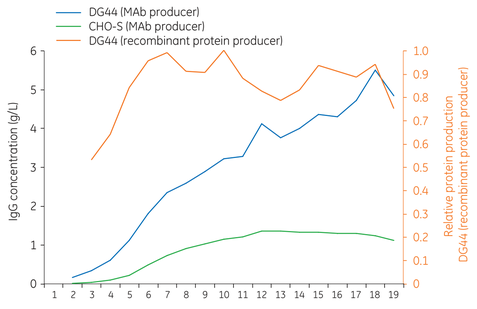

Fig 8. Productivity in 2 L ActiPro fed-batch bioreactor cultures.

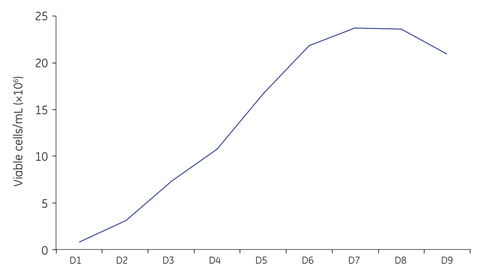

Fig 9. DG44 cell growth in 50 L ActiPro bioreactor cultures.

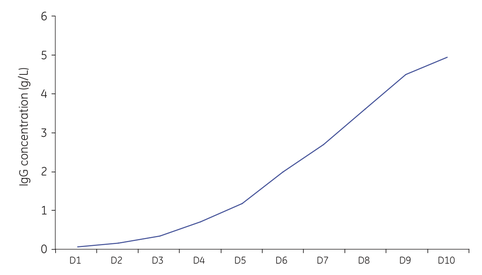

Fig 10. DG44 cell productivity in 50 L ActiPro bioreactor cultures.

Conclusion

For the selected CHO cell clones, ActiPro medium and supplements support higher viable cell densities and protein production compared with other media and supplements included in this study. ActiPro medium and Cell Boost supplements allow for easy scale-up, from benchtop to production bioreactor runs.

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