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Research Report

Extended, High Density Growth of CHO-K1 Cells in EX-CELL® 302 Serum-Free Medium

Introduction

SAFC® has developed a serum-free medium, EX-CELL 302, which meets or exceeds requirements for use with CHO-K1 cells grown in suspension culture. Raw materials (amino acids, lipid supplements and carbohydrates) used in this medium are of the highest quality: USP, EP, JP or ACS, and are non-animal sourced wherever possible. With the addition of 4 mM L-glutamine, EX-CELL 302 will support growth of CHO-K1 cells in batch suspension cultures for greater than eight (8) days while maintaining high cell viability (> 70%). This long culture period and stationary phase allow for an extended production period and higher culture productivity. L-glutamine, hypoxanthine and thymidine have been omitted from EX-CELL 302 allowing for use with either the DHFR- or GS selection systems.

Materials and Methods

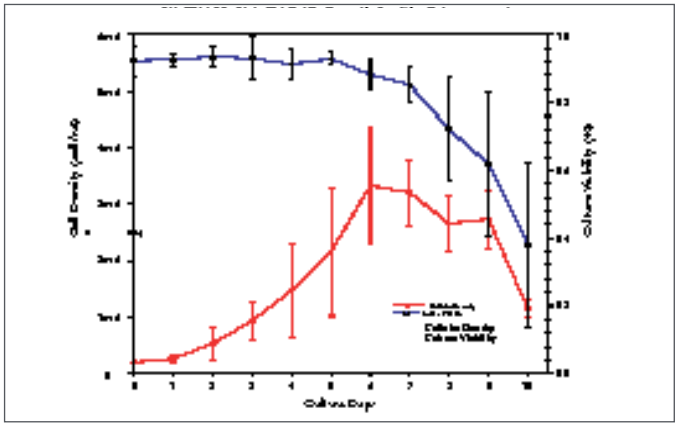
- CHO-K1 cells (ATCC CCL 61) were obtained from the American Type Culture Collection (Rockville, MD) and were previously adapted to EX-CELL 301 (Catalog No. 14331C-Available for custom development only).
- L-Glutamine (Catalog No. 59202C), SAFC, Lenexa, Kansas.
- EX-CELL 302 (Catalog No. 14312C-Available for custom development only), SAFC, Lenexa, Kansas.
- EX-CELL 301 (Catalog No. 14311C-Available for custom development only), SAFC, Lenexa, Kansas.

Cell Culture

- Cultures were maintained in an incubator in 250 mL Bellco spinner flasks using a seeding density of 2 x 10^5 cells/mL in 100 mL of EX-CELL 302. Impeller speed was set to approximately 60 rpm.
- Daily cell counts and viabilities were determined by Trypan Blue dye exclusion.

Results

Figure 1. EX-CELL 302 CHO-K1 Growth Profile (10 passages)



CHO-K1 cultures were monitored daily for growth and viability. These data represent the average cell density and viability for 10 passages. Cultures were seeded at a density of 2 x 10^5 cells/mL in 100 mL media using a spinner speed of 60 rpm. An increase in growth rate and final cell density were seen in subsequent passages after full adaptation.

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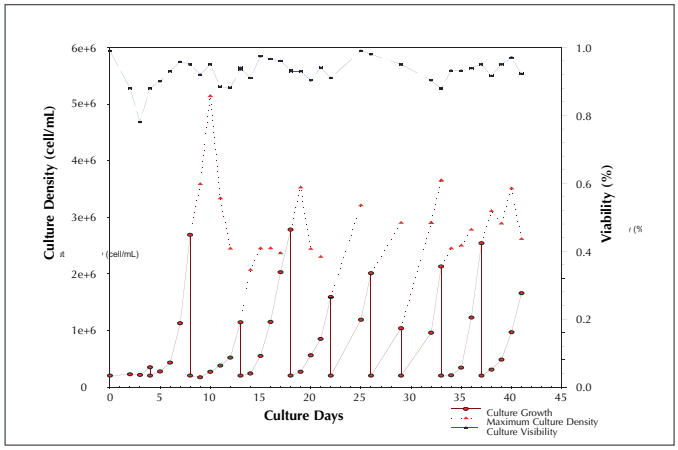
Figure 2: EX-CELL® 302 CHO-K1 Multiple Passage



Spinner cultures were initiated from stationary cell cultures that were grown in EX-CELL 301 medium. Cultures were passaged every 4 - 5 days using a seeding density of 2 x 10^5 cells/mL. Cultures were monitored daily for maximum cell density after passaging. Cultures were terminated when culture viability dropped below 70%.

Conclusions

Data presented here demonstrate extended cellular growth in EX-CELL 302 medium for eight (8) days in spinner batch cultures. Cultures of CHO-K1 cells achieved an average maximum cell density of approximately 3.5 x 10^6 cells/mL with exponential doubling times in the range of 20 hours while maintaining viabilities greater than 70%. EX-CELL 302 contains low levels (< 100 μg/L total) of recombinant proteins. CHO-K1 cells, cryopreserved and adapted to serum-free growth in EX-CELL 301, were transferred directly into EX-CELL 302 without an adaptation period. SAFC's regulatory friendly EX-CELL 302 is suitable for the production of human therapeutic products since it does not contain animal-derived proteins or other undesirable components.

For more information, visit safcglobal.com or contact SAFC

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