

# Research Report

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

### Introduction

SAFC Biosciences 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<sup>r</sup> or GS selection systems.

### Materials and Methods

#### Materials

- 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. 14331).
- L-Glutamine (Catalog No. 59202), SAFC Biosciences, Lenexa, Kansas.
- EX-CELL™ 302 (Catalog No. 14312), SAFC Biosciences, Lenexa, Kansas.
- EX-CELL™ 301 (Catalog No. 14311), SAFC Biosciences, Lenexa, Kansas.

### Cell Culture:

- Cultures were maintained in an incubator in 250 mL Bellco spinner flasks using a seeding density of  $2 \times 10^5$  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

#### EX-CELL™ 302 CHO-K1 Growth Profile (10 passages)

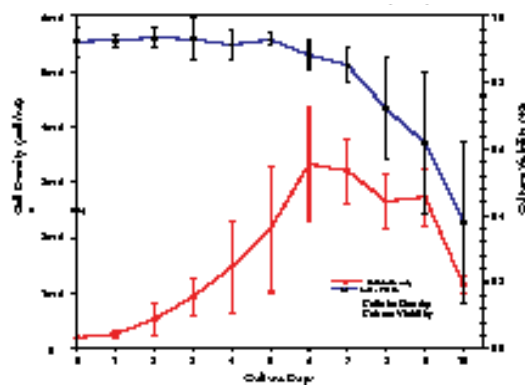


Figure 1: Figure 1. CHO-K1 cultures were monitored daily for growth and viability. These data represent the average cell density and viability for 10 passages. Cultures were seeded at a density of  $2 \times 10^5$  cells/mL in 100 mL media using a spinner speed of 60 rpm. An increase in growth rate and final cell density were seen in subsequent passages after full adaptation.

#### United States

SAFC Biosciences, Inc.  
13804 W. 107th Street  
Lenexa, Kansas 66215  
USA  
Phone +1 913-469-5580  
Toll free-USA 1 800-255-6032  
Fax +1 913-469-5584  
E-mail info-na@sial.com

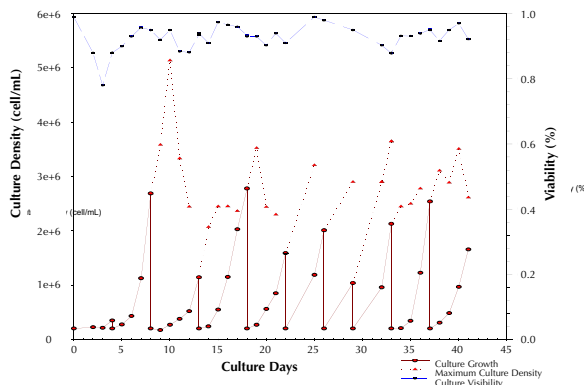
#### Europe

SAFC Biosciences Ltd.  
Smeaton Road, West Portway  
Andover, Hampshire SP10 3LF  
UNITED KINGDOM  
Phone +44 (0)1264-333311  
Fax +44 (0)1264-332412  
E-mail info-eu@sial.com

#### Asia Pacific

SAFC Biosciences Pty. Ltd.  
18-20 Export Drive  
Brooklyn, Victoria 3025  
AUSTRALIA  
Phone +61 (0)3-9362-4500  
Toll free-AUS 1 800-200-404  
Fax +61 (0)3-9315-1656  
E-mail info-ap@sial.com

## EX-CELL™ 302 CHO-K1 Multiple Passage



**Figure 2:** 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  $2 \times 10^5$  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  $3.5 \times 10^6$  cells/mL with exponential doubling times in the range of 20 hours while maintaining viabilities greater than 70%. EX-CELL™ 302 contains low levels ( $< 100 \mu\text{g/L}$  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 Biosciences' 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.

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### United States

SAFC Biosciences, Inc.  
13804 W. 107th Street  
Lenexa, Kansas 66215  
USA  
Phone +1 913-469-5580  
Toll free-USA 1 800-255-6032  
Fax +1 913-469-5584  
E-mail info-na@sial.com

### Europe

SAFC Biosciences Ltd.  
Smeaton Road, West Portway  
Andover, Hampshire SP10 3LF  
UNITED KINGDOM  
Phone +44 (0)1264-333311  
Fax +44 (0)1264-332412  
E-mail info-eu@sial.com

### Asia Pacific

SAFC Biosciences Pty. Ltd.  
18-20 Export Drive  
Brooklyn, Victoria 3025  
AUSTRALIA  
Phone +61 (0)3-9362-4500  
Toll free-AUS 1 800-200-404  
Fax +61 (0)3-9315-1656  
E-mail info-ap@sial.com