

Research Report

HEK 293 Cell Growth and Virus Production in EX-CELL[®] 293 Serum-Free Medium

Manisha Sahni, Shelley Wilcox, Pamela Mettner, Cynthia Reiss, Sarah L. Gilliland, Douglas R. Purtle, Karen J. Etchberger

Abstract

The Human Embryonic Kidney (HEK) 293 cell line is used in the development of viral vaccines, anticancer agents and the production of recombinant adenoviral vectors. EX-CELL 293 is a serum-free, animal-protein free medium designed and optimized to support high-density suspension culture of HEK 293 cells. The HEK 293 cell line easily adapted to EX-CELL 293 without forming cellular aggregates and achieved average cell densities of 2.5×10^6 cells/mL in shaker flasks, with an approximate doubling time of 33 hours. HEK 293 cells in EX-CELL 293 were also infected with wild-type Adenovirus type 5 (Ad5) and produced $1-3 \times 10^{10}$ TCID₅₀/mL. We conclude that EX-CELL 293 supports rapid HEK 293 cell growth in shaker flasks and produces high density, highly viable cultures that are capable of adenovirus production.

Introduction

The HEK 293 cell line is an attractive instrument in the field of gene therapy. The cell line is capable of producing glycosylated human proteins and acts as a host for the production of recombinant adenoviral vectors, which have shown promise in treating diseases such as cystic fibrosis, hypertension and arthritis. Traditionally, HEK 293 cells are grown as attachment cultures in a serum-supplemented basal medium such as Dulbecco's Modified Eagle's Medium (DMEM). EX-CELL 293 is a serum-free, animal-protein free medium specifically formulated to support large-scale, high-density suspension culture and virus production in the HEK 293 cell line. The medium contains very low levels of recombinant protein (approximately 1.1 mg/L), facilitating downstream processing of expressed products and eliminating regulatory concerns associated with serum and animal proteins. Additionally, the liquid formulation of EX-CELL 293 is formulated without L-glutamine, which avoids problems associated with L-glutamine degradation and improves product shelf-life. Our experiments show EX-CELL 293 supports high-density, serum-free HEK 293 cell growth and supports the production of high yields of adenovirus.

Materials

Cells

- HEK 293 cell line, American Type Culture Collection, Catalog No. CRL-1573

Virus

- Wild-type Adenovirus Type 5, American Type Culture Collection, Catalog No. VR-5

Serum-Free Media

- EX-CELL 293, SAFC, Catalog No. 14570C. L-glutamine was aseptically added to EX-CELL 293 to a final concentration of 6 mM. No antibiotics or other supplements were added to the medium.

Other Media and Supplements

- 200 mM L-glutamine, SAFC, Catalog No. 59202C
- Dulbecco's Modified Eagle's Medium (DMEM)/High Modified, SAFC, Catalog No. 51444C
- Fetal Bovine Serum (FBS) - Gamma Irradiated, SAFC, Catalog No. 12107C
- 0.25% Trypsin (1X) 0.1% EDTA Solution, SAFC, Catalog No. 59229C (available for custom development only)

Methods

Adaptation and Growth Studies

HEK 293 cells were adapted to EX-CELL 293 by direct adaptation. As such, adherent cultures of HEK 293 cells in DMEM/High Modified supplemented with 5% FBS were trypsinized and counted. Cells were subsequently seeded (6×10^5 cells/mL) directly into pre-warmed EX-CELL 293 media (35 mL volume per 125 mL shaker flasks). Flasks were incubated with loosened caps at 37 C in a 10% CO₂ atmosphere, with an orbital shaking speed of 90 - 100 rpm. Cultures were passaged twice per week (5×10^6 cells/mL) and were considered fully adapted after 6 passages, as indicated by sustained viabilities above 90%, and at least one cell division per passage. Cell densities and viabilities were determined by trypan blue exclusion.

Growth of HEK 293 cells was monitored for more than 15 passages in EX-CELL® 293. Cultures were maintained in shaker flasks using the same culture conditions as above. Additionally, growth characteristics in unfed cultures were examined in shaker flask cultures by observing cell growth over a period of time (10 days).

Ad5 Viral Infection and TCID₅₀ Titration

Three independent infections with wild-type Ad5 were performed in EX-CELL 293. Cells were passed on day 4 at a seeding density of 5×10^5 cells/mL, and infected on day 2 at approximately 1×10^6 cells/mL with wild-type Ad5 at a multiplicity of infection (MOI) of 5. Samples were collected at 24, 48, 72 and 96 hours and were subjected to 3 rounds of freezing/thawing (-70 C to 37 C). Each lysate was passed through a 0.22 μ m sterile syringe filter and stored frozen (-70 C) until titered.

Titration was performed in 96-well microtiter plates as follows: HEK 293 cells were seeded in DMEM with 6 mM L-glutamine and 5% FBS at 1×10^4 cells/100 μ L/well. Duplicate serial dilutions (10^{-1} to 10^{-11}) of each lysate were made in DMEM with 2% FBS and 2 mM L-glutamine, and 100 μ L was added to each well. Plates were incubated at 37 C, 10% CO₂ for 10 days, then observed on an inverted microscope for cytopathic effect (CPE). The titer (T) was determined as follows:

$$T = 10^{1+d(S-0.5)}$$

Where d = Log₁₀ of the dilution and S = Sum of the ratios.

Results

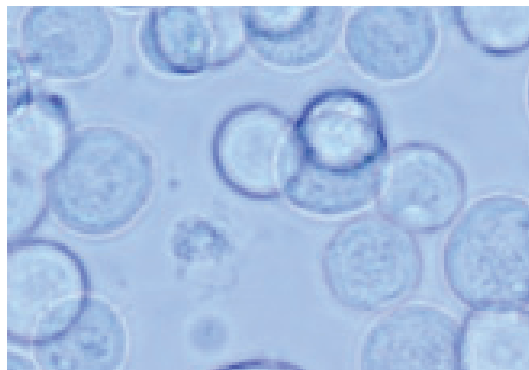
HEK 293 cells were adapted to EX-CELL 293 by direct adaptation, i.e. a complete medium exchange from serum-containing DMEM to 100% serum-free medium. The cells adapted quickly without experiencing a significant lag phase and without forming cellular aggregates or clumps (see Figure 1).

Figure 2 depicts the growth of HEK 293 cells cultured in EX-CELL 293 in shaker flasks over 16 passages. The average cell density reached per passage was 2.5×10^6 cells/mL (range 1.4 - 4.1×10^6 cells/mL) and the average cell doubling time was 33 hours (range 24 - 45 hours). Culture viability was typically above 98% and was greater than 95% for all passages.

Figure 3 illustrates a typical growth pattern of HEK 293 cells in unfed shaker cultures over a period of 10 days. EX-CELL 293 supported exponential cell growth for 8 days with viabilities above 90%.

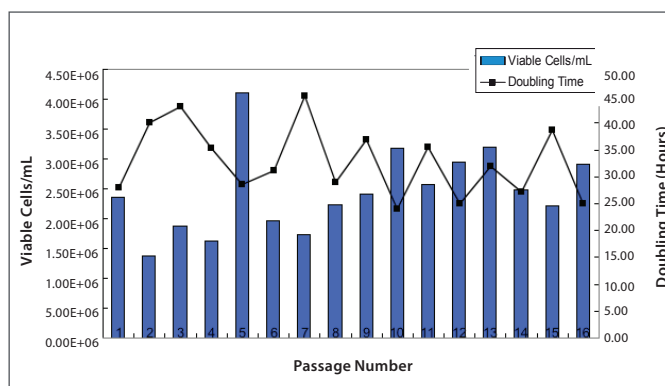
Preliminary studies determined the optimal time of infection and MOI (i.e., 48 hours after seeding at a MOI of 5) for HEK 293 cells in EX-CELL 293. Using these parameters, HEK 293 cells grown in EX-CELL 293 supported the production of wild-type Ad5 with yields in the range of 3×10^{10} TCID₅₀/mL.

Figure 1. HEK 293 cells in EX-CELL 293



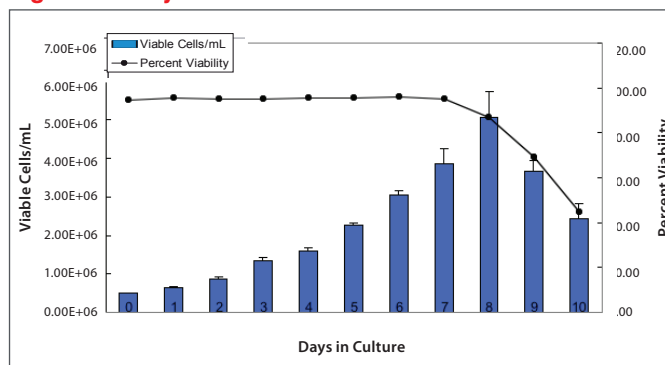
EX-CELL 293 retards HEK 293 cellular aggregation, allowing for easier culture, increased culture viability and greater product yield.

Figure 2. Typical Growth of HEK 293 Cells in EX-CELL 293 in Shaker Flasks



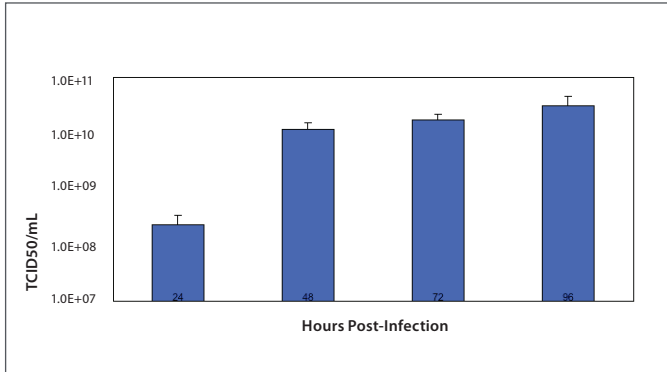
HEK 293 cells were seeded at 4×10^5 cells/mL in 125 mL shaker flasks containing 35 mL of EX-CELL 293. Flasks were incubated in a 10% CO₂ atmosphere at 37 C, with a shaker speed of 90 - 100 rpm and were subcultured every 3 - 4 days.

Figure 3. Daily Growth of HEK 293 cells in EX-CELL 293



HEK 293 cells were seeded at 4×10^5 cells/mL in 125 mL shaker flasks containing 35 mL of EX-CELL 293. Flasks were incubated in a 10% CO₂ atmosphere at 37 C, with a shaker speed of 90 - 100 rpm, for 10 days without refeeding.

Figure 4. Titration (TCID₅₀/mL) of Ad5 Produced by HEK 293 Cells in EX-CELL® 293



HEK 293 cells were infected with wild-type Ad5 48 hours post-seeding at a MOI of 5. Samples were collected at 24, 48, 72 and 96 hours post-infection. Results represent TCID₅₀/mL at each time point.

Conclusions

- HEK 293 cells easily adapted to suspension culture in EX-CELL 293 with very little cellular aggregation.
- EX-CELL 293 produced high-density, highly viable HEK 293 cultures with up to 5 x 10⁶ viable cells/mL.
- EX-CELL 293 supports highly viable HEK 293 cultures with most cultures above 98% viability.
- HEK 293 cells grown in EX-CELL 293 produce high yields of adenovirus, up to 3 x 10¹⁰ TCID₅₀/mL.
- EX-CELL 293 is serum-free, animal-protein free and contains very low levels of recombinant protein, facilitating downstream purification of products.
- EX-CELL 293 is formulated without L-glutamine, thereby increasing product stability and shelf life.

For more information, visit safcglobal.com or contact SAFC

USA: + 1-913-469-5580 • info-na@sial.com

Europe: +44 (0)1264-333311 • info-eu@sial.com

Asia/Pacific: +61 (0)2 9841 0555 • auscustserv@sial.com

Warranty, Limitation of Remedies

SAFC warrants to the purchaser for a period of one year from date of delivery that this product conforms to its specifications. Other terms and conditions of this warranty are contained in SAFC written warranty, a copy of which is available upon request. ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING THE IMPLIED WARRANTY OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, ARE EXCLUDED. In no case will SAFC be liable for any special, incidental, or consequential damages arising out of this product or the use of this product by the customer or any third party based upon breach of warranty, breach of contract, negligence, strict tort, or any other legal theory. SAFC expressly disclaims any warranty against claims by any third party by way of infringement or the like. THIS PRODUCT IS INTENDED FOR PURPOSES DESCRIBED ONLY AND IS NOT INTENDED FOR ANY HUMAN OR THERAPEUTIC USE.

Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

©2012 Sigma-Aldrich Co. LLC. All rights reserved. SAFC and SIGMA-ALDRICH are trademarks of Sigma-Aldrich Co. LLC, registered in the US and other countries. EX-CELL is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the SAFC website at www.safcglobal.com and/or on the reverse side of the invoice or packing slip.

Issued June 2012 R022
 0103 0206