

Technical Bulletin

EX-CELL™ Vero Serum-Free Medium for High-Density Vero Growth

Introduction

The Vero cell line, isolated from the kidney of a normal adult African Green monkey (*Cercopithecus aethiops*), has been well characterized and is instrumental in the biotechnology sector for virus replication studies, viral plaque assays, TCID₅₀ determinations and production of viral vaccines.

SAFC Biosciences has developed EX-CELL™ Vero Serum-Free Medium for Vero Cells specifically for the Vero cell line. EX-CELL™ Vero is free of animal-derived components. The medium contains a plant-derived hydrolysate and low levels of recombinant proteins, but does not contain phenol red or Pluronic® F68. In these studies, we show that EX-CELL™ Vero supports high-density cell growth in both stationary flasks and on microcarriers in bioreactor culture.

Experimental Design

The following study was designed to assess the growth of Vero cells in EX-CELL™ Vero. We evaluated:

- Direct adaptation to EX-CELL™ Vero from serum-supplemented basal medium;
- Adaptation to EX-CELL™ Vero from serum-free competitor media: VP-SFM (GIBCO™) and HyQ® SFM4MegaVir™ (HyClone);
- Growth (morphology, cell densities, doubling times and viabilities) over multiple passages in T-flasks; and
- Growth in stirred tank bioreactors using microcarriers.

Materials

Cells

- Vero, *Cercopithecus aethiops* (monkey, African green), American Type Culture Collection, ATCC No. CCL-81

SAFC Biosciences Media and Supplements

- EX-CELL™ Vero Serum-Free Medium for Vero Cells, Catalog No. 14585
- Dulbecco's Modified Eagle's Medium/High Modified (DMEM/High), Catalog No. 51444
- Fetal Bovine Serum (FBS), Catalog No. 12103
- L-Glutamine Solution 200 mM, Catalog No. 59202
- Trypsin-EDTA Solution 1X, 0.25% trypsin, 0.1% EDTA, trypsin gamma irradiated by SER-TAIN™ Process, Catalog No. 59429
- Dulbecco's Phosphate Buffered Saline (DPBS Modified), Catalog No. 59321

Other Media and Supplements

- VP-SFM, GIBCO™, Catalog No. 11681-020
- HyQ® SFM4MegaVir™, HyClone, Catalog No. SH30553.02
- Trypsin inhibitor from Glycine max (soybean) (STI), Sigma-Aldrich Co., Product No. T6522

Bioreactor Supplies

- 5 L stirred tank bioreactor, Applikon, Inc.
- Cytodex™ 1 microcarriers, Amersham Biosciences, Catalog No. 17-0448
- Crystal Violet, Sigma-Aldrich, Product No. C3886

Methods

Media Preparation

All media were stored at 4 C in the dark and were supplemented with 4 mM L-glutamine at time of use. For bioreactor studies, 0.05% Pluronic® F68 was added to the media. Cultures were maintained using aseptic technique; antibiotics and fungicides were not used.

Culture Techniques

T-Flasks:

To trypsinize the cells, spent media was aspirated and the cells rinsed with DPBS Modified. Trypsin was added to the flask and the cultures incubated at 37 C for 3 - 5 minutes until the cells dissociated. An excess volume of STI (1 mg/mL) was added to the flask, and the volume adjusted

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to 10 mL with fresh medium. The cells were transferred to centrifuge tubes and centrifuged for five minutes at 200 g. The supernatant was aspirated and the cells resuspended in 10 mL fresh medium. Cell density and viability were determined using a Cedex Cell Counter (AS20, Innovatis) and trypan blue exclusion. Cells were transferred to new vessels and placed in a humidified incubator at 37 C with 5% CO₂.

Bioreactors:

Vero cells were inoculated in Applikon stirred tank bioreactors in a working volume of 3 L of medium. Cytodex™ 1 microcarriers were hydrated, sterilized and acclimatized to each medium per the manufacturer's directions. Cells were seeded at 20 cells per microcarrier (approximately 4 x 10⁵ cells/mL); the total concentration of microcarriers was 3 g/L. Reactor temperature was set to 37 C, the agitation speed was 70 - 85 rpm, dissolved O₂ was maintained at 50% and pH was maintained at 7.0 - 7.3 with CO₂. Reactors were monitored and samples obtained daily for seven days; cell growth was monitored by counting released nuclei using a crystal violet staining procedure. Growth in bioreactors was evaluated only in EX-CELL™ Vero and VP-SFM. Several attempts were made to initiate bioreactor cultures in HyQ® SFM4MegaVir™, however we were unsuccessful in getting the cells to adhere to the microcarriers in this medium.

Results

Direct Adaptation from DMEM/Modified containing 5% FBS

Vero cells that were maintained in DMEM/High with 5% FBS were trypsinized and seeded in triplicate 75 cm² T-flasks at 5 x 10⁴ cells/cm² in a total volume of 20 mL EX-CELL™ Vero, VP-SFM and HyQ® SFM4MegaVir™ (supplemented with 4 mM L-glutamine). The flasks were subcultured every three days for five passages, then the seeding density was reduced to 2 x 10⁴ cells/cm² (maintenance seeding density) and the flasks were passaged every four days. Table 1 indicates the average cell densities, doubling times and viabilities obtained in each medium during the course of the study. Figure 1 illustrates the growth in each medium over 10 passages during adaptation from serum-containing basal medium to each respective serum-free medium.

Table 1

Medium	Average Cell Density (cells/cm ² x 10 ⁵)	Average Doubling Time Hours	Average Percent Viability
EX-CELL™ Vero	2.47 (± 0.2)	29.0 (± 1.0)	98.3 (± 0.4)
VP-SFM	1.81 (± 0.2)	34.1 (± 2.1)	97.8 (± 0.6)
HyQ® SFM4MegaVir™	1.42 (± 0.1)	40.5 (± 1.5)	98.5 (± 0.6)

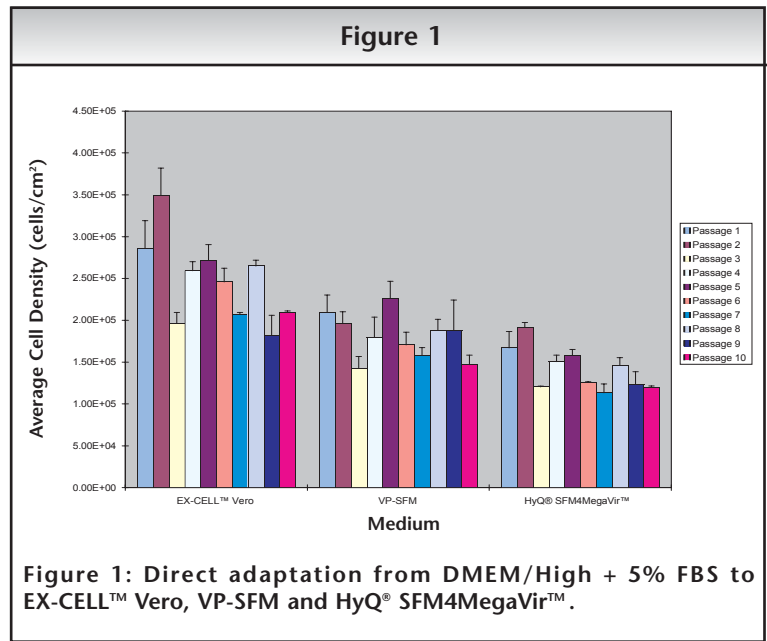


Figure 1: Direct adaptation from DMEM/High + 5% FBS to EX-CELL™ Vero, VP-SFM and HyQ® SFM4MegaVir™.

Growth Curves

Fully adapted Vero cells were seeded in each medium in 25cm² T-flasks at 2 x 10⁴ cells/cm² (triplicate flasks for each day) and growth was determined daily for seven days. Figure 2 illustrates typical growth curves in each respective medium. Culture viabilities remained above 95% in all media during the study.

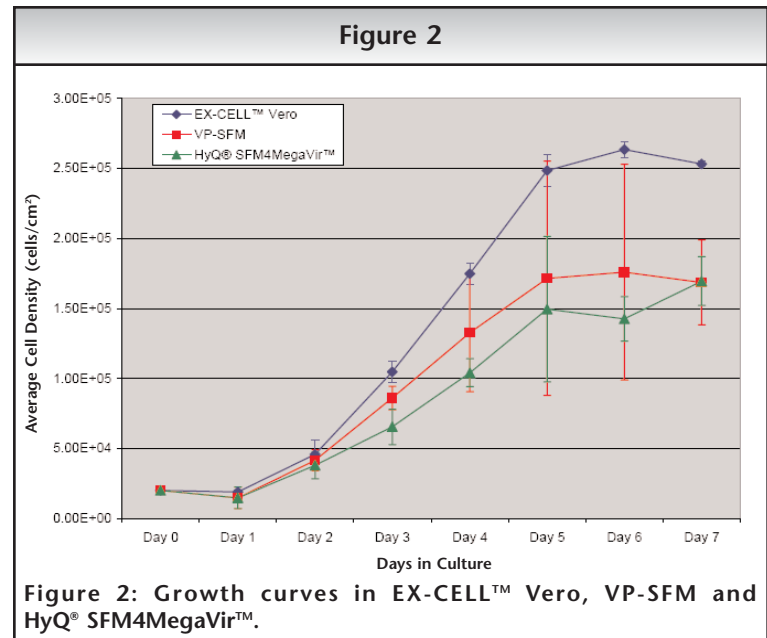
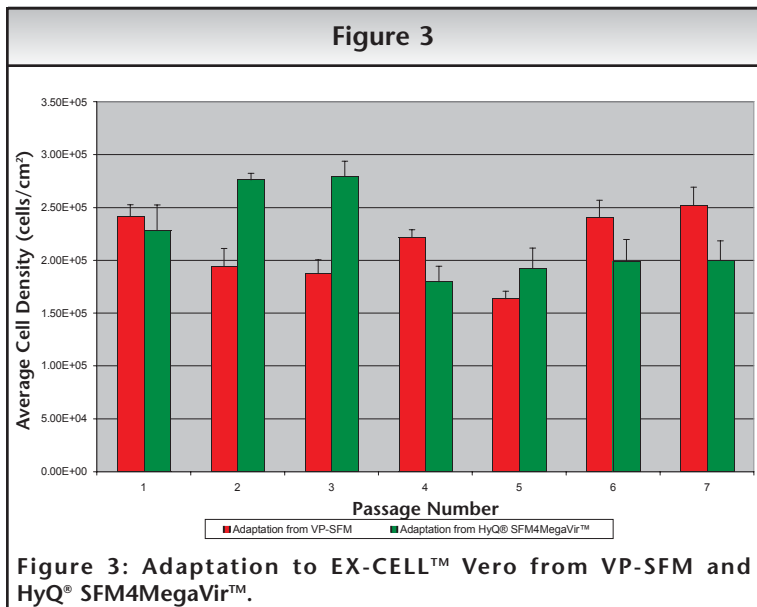


Figure 2: Growth curves in EX-CELL™ Vero, VP-SFM and HyQ® SFM4MegaVir™.

Direct Adaptation to EX-CELL™ Vero from Competitor Serum-Free

Vero cells which were fully adapted to VP-SFM and HyQ® SFM4MegaVir™ were used in this study. Cultures were trypsinized as described above and subcultured directly into EX-CELL™ Vero at 2×10^4 cells/cm². The cells were passaged every four days for at least seven passages. The average cell density in EX-CELL™ Vero was comparable to the cell density observed during the adaptation study (see Table 1).



Growth in Bioreactors

Vero cells attached well to the Cytodex™ 1 microcarriers, without forming aggregates. Cells grew to confluence (see Figure 5) and achieved maximum densities of approximately 1.9×10^6 cells/mL ($\sim 1.5 \times 10^5$ cells/cm²) in EX-CELL™ Vero and 1.4×10^6 cells/mL ($\sim 1.1 \times 10^5$ cells/cm²) in VP-SFM (see Figure 4).

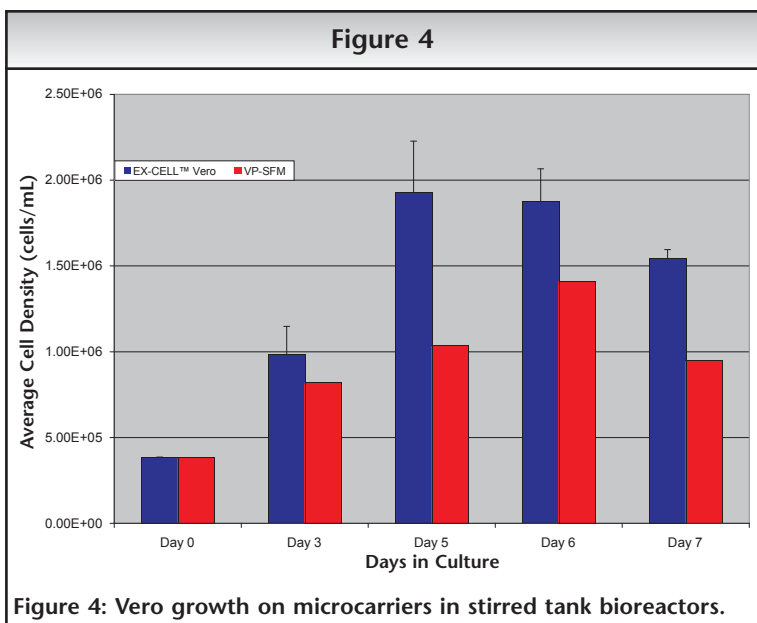


Figure 5

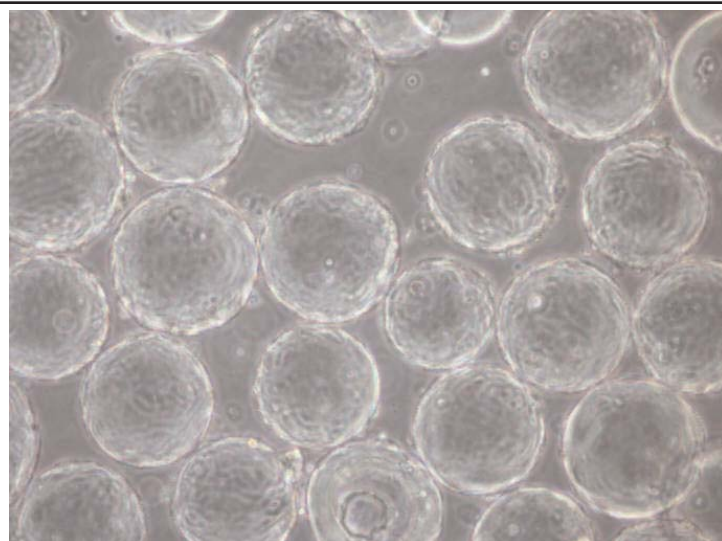


Figure 5: Vero cells attached to microcarriers in EX-CELL™ Vero.

Conclusions

EX-CELL™ Vero is a serum-free medium designed and optimized for high-density Vero cell growth in adherent-stationary and adherent-suspension conditions. EX-CELL™ Vero is a regulatory-compliant medium, free from all animal-derived components and contains only recombinant proteins. These studies indicate that: adaptation to EX-CELL™ Vero from both basal medium and serum-free medium is quickly and easily accomplished; EX-CELL™ Vero supports high-density Vero cell growth; and, growth in EX-CELL™ Vero is superior to growth in competitor formulations.

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