

Research Report

Evaluation of Virus Production in Vero Cells Grown in EX-CELL™ Vero Serum-Free Medium

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Abstract

EX-CELL™ Vero Serum-Free Medium for Vero Cells is an animal-component free, low-protein medium designed and optimized to support high density cultures of African Green Monkey Kidney (Vero) cells. The Vero cell line can be easily adapted to EX-CELL™ Vero using a direct adaptation method. EX-CELL™ Vero supports Vero cell density up to 2.5×10^5 cells/cm² with doubling time as short as 29 hours. Vero cells in EX-CELL™ Vero were infected with Herpes Simplex Virus-Type 2 (HSV-2) and produced on average $10^{6.0}$ TCID₅₀/mL at a multiplicity of infection (MOI) of 0.01. It was concluded that EX-CELL™ Vero supports rapid Vero cell growth and produces HSV-2 titers comparable to serum-supplemented cultures.

Introduction

The Vero cell line is used in applications such as development of viral vaccines and the production of recombinant adenoviral vectors. Traditionally, Vero cells are grown in serum-supplemented basal medium such as Dulbecco's Modified Eagle's Medium (DMEM). EX-CELL™ Vero is an animal-component free, low-protein, serum-free medium specially formulated to support large-scale, high-density Vero culture and virus production. The medium contains very low levels of recombinant protein (approximately 0.5 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™ Vero is formulated without L-glutamine, which avoids problems associated with L-glutamine degradation, therefore improving product shelf life. Our experiments show that EX-CELL™ Vero supports high-density, serum-free Vero cell growth and the production of virus yields comparable to serum-supplemented cultures.

Materials

Cells

- African Green Monkey Kidney cell line, American Type Culture Collection, Catalog No. CCL-81

Virus

- Herpes Simplex Virus 2 (HSV-2), American Type Culture Collection, Catalog No. VR-540

Serum-Free Media

- EX-CELL™ Vero, SAFC Biosciences, Catalog No. 14585

Other Media and Supplements

- Dulbecco's Modified Eagle's Medium/High Modified (DMEM/High), SAFC Biosciences, Catalog No. 51444
- Fetal Bovine Serum (FBS) Gamma Irradiated, SAFC Biosciences, Catalog No. 12107
- L-Glutamine Solution 200mM, SAFC Biosciences, Catalog No. 59202
- Trypsin-EDTA Solution 1X, 0.25% trypsin, 0.1% EDTA, trypsin gamma irradiated by SER-TAIN™ Process, SAFC Biosciences, Catalog No. 59429
- Dulbecco's Phosphate Buffered Saline (DPBS Modified), SAFC Biosciences, Catalog No. 59321
- Trypsin inhibitor from Glycine max (soybean) (STI), Sigma-Aldrich Co., Product No. T6522

Methods

EX-CELL™ Vero was prepared by adding L-glutamine at a final concentration of 4 mM at time of use. Unless otherwise noted, all EX-CELL™ Vero was supplemented before use. 5% FBS was added to DMEM/High at time of use (referred to as DMEM-5%). STI was prepared as a concentrated (10 mg/mL) solution in DPBS and filter sterilized (0.2 µm). Working stock solutions of STI were diluted to 1 mg/mL with sterile DPBS as needed. All media were stored at 2 to 8 C, protected from light. Other supplements were stored at their recommended temperatures.

Basic Culture Techniques

Cells were routinely subcultured every four days (96 hours ± 6 hours) at a seeding density of 2×10^4 cells/cm² in EX-CELL™ Vero and DMEM-5% in 75 cm² vent cap T-flasks. The total volume of media was 20 mL per flask. Cultures were maintained at 37 ± 1 C in a humidified incubator with 5% CO₂. All experiments were performed in duplicate flasks and cell counts were determined using a Cedex counter. All cultures were maintained using aseptic technique and without the use of antibiotics or fungicides.

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Trypsinization

Vero cultures in EX-CELL™ Vero and DMEM-5%:

Spent medium was aspirated and the monolayer rinsed with 5 mL DPBS. The DPBS was aspirated and 1 mL trypsin was added to the flasks. The flasks were incubated at 37 C for 3 - 5 minutes until the cells dissociated. 4.5 mL of STI was then added to the flasks to neutralize the trypsin, the cells were gently resuspended in 4.5 mL of the respective medium and cultures were placed in a 15 mL conical tube. Cultures were centrifuged at 228 *g* for five minutes to pellet the cells. The supernatant was removed, discarded and the pellet resuspended in 10 mL of the respective medium. A 1 mL aliquot was taken for counting on the Cedex and cells were subsequently diluted in the appropriate quantity of prepared medium and incubated as above.

Notes:

1. Vero cells in both media appeared sensitive to trypsin. It was found that using a lower volume of STI did not fully inactivate the trypsin and caused the cells to exhibit an unusual morphology.
2. Over-trypsinizing the cells may occur quickly. It is important to inactivate the trypsin within 15 minutes (less time in smaller flasks). Over-trypsinized cells do not recover.

Direct Adaptation

Vero cultures were started from frozen cells in DMEM-5% in 25 cm² T-flasks and were expanded and maintained in 75 cm² T-flasks. Vero cells were subcultured directly into EX-CELL™ Vero at a seeding density of 2 x 10⁴ cells/cm². The cells reached 100% confluency within three days and displayed normal doubling times.

Herpes Simplex Virus-2 Production

To determine the capability of EX-CELL™ Vero to support the production of virus, Vero cells were infected with Herpes Simplex Virus-2 (HSV-2). Vero cells were seeded in EX-CELL™ Vero and DMEM-5% in 25 cm² T-flasks at 2 x 10⁴ cells/cm² in a total volume of 5 mL medium per flask. Flasks were incubated at 37 C with 5% CO₂. Once the flasks were confluent (Figure 1 and Figure 2), one flask of each condition was sacrificed and the total cell count was determined. The cells were then infected with the appropriate amount of virus to yield a MOI of 0.01. Flasks were incubated as above and during the infection the cells were monitored and displayed cytopathetic effect (CPE). The cells became rounded and many, although not all, of the cells detached from the bottom of the flask. The flasks were harvested when greater than 75% CPE was observed, which was Day 4 (Figure 3 and Figure 4). Prior to titration, each flask was subjected to three rounds of thawing (37 C) and freezing (-70 C) to lyse the cells. Any remaining attached cells were sloughed off the flask by rapping the flasks against the palm of the hand and by pipetting the lysate forcefully against the bottom of the flask. The cell lysates were transferred to 15 mL conical tubes and centrifuged at ~2000 *g* for 10 minutes to pellet the cell debris. The supernatants were transferred to new tubes and titrated.

Figure 1

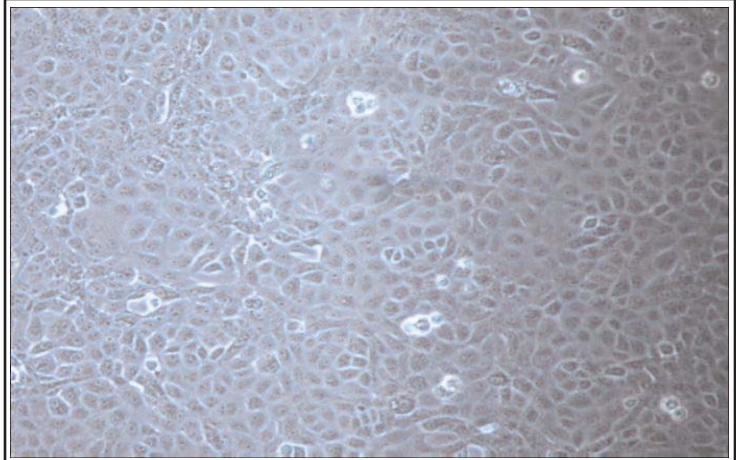


Figure 1: Point of Infection of Vero cells in EX-CELL™ Vero (Day 4).

Figure 2

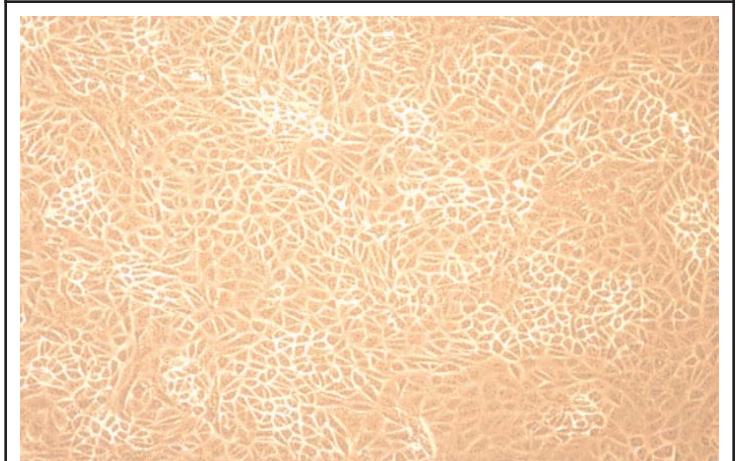


Figure 2: Point of infection of Vero cells in DMEM-5% (Day 4).

Figure 3

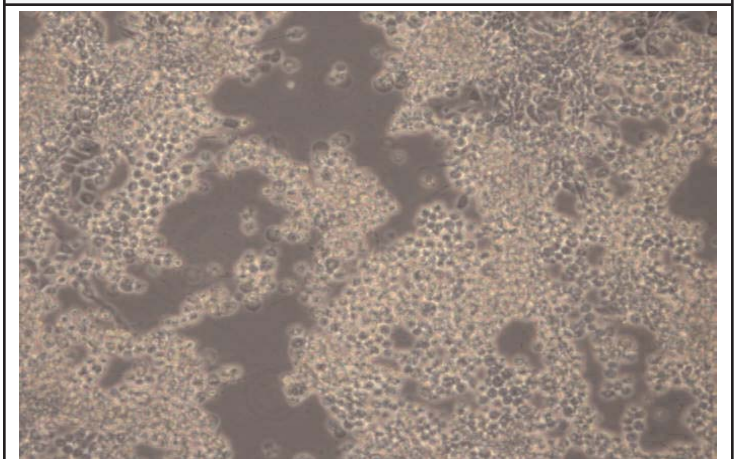


Figure 3: Day 4 of Vero cells infected with HSV-2 in DMEM-5%. This was the point of harvest (Day 4 post-infection).

Figure 4

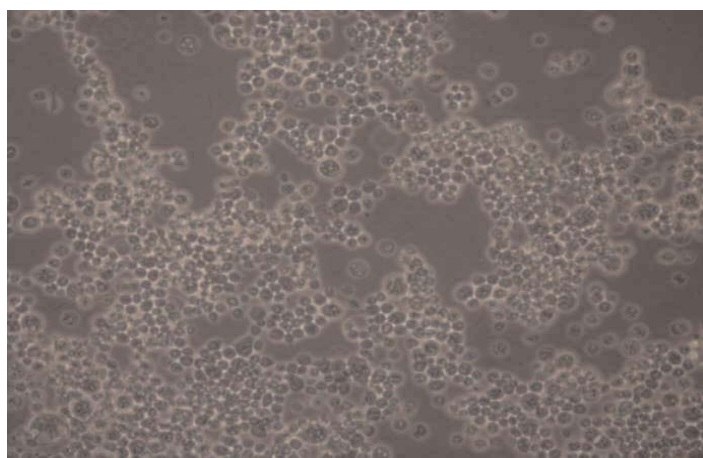


Figure 4: Day 4 of Vero cells infected with HSV-2 in EX-CELL™ Vero. This was the point of harvest (Day 4 post-infection).

TCID₅₀/mL Titrations

HSV-2 production in EX-CELL™ Vero was determined by TCID₅₀. Vero cells growing in DMEM-5% were harvested by trypsinization, counted and diluted to a final concentration of 2×10^5 cells/mL in DMEM supplemented with 5% FBS (DMEM-5%). Using a 12-channel pipettor, 100 μ L of cells were dispensed into 96-well microtiter plates (2×10^4 cells/well) and allowed to attach at 37 C, 5% CO₂ for two days. Duplicate serial dilutions of each lysate were made in DMEM-2% in sterile snap-cap disposable

tubes (1:10 dilutions, 10^{-4} to 10^{-8} plated). The spent medium on the 96-well plates was decanted into a sterile container under the laminar flow hood. The dilutions were plated in duplicate on each plate in wells 1 - 10 (wells 11 and 12 in all rows served as negative controls). The rows were dispensed with the highest (10^{-8}) dilution in column 10 and the lowest dilution (10^{-4}) in column 6. The second dilution of each set was dispensed in columns 5 to 1. The plates were incubated at 37 C, 5% CO₂ for three days and then observed on an inverted microscope for CPE. Wells were considered positive even if only a small area of the well showed CPE. The titer was determined using the Reed and Muench statistical method¹.

Conclusions

Vero cells were adapted from DMEM-5% to EX-CELL™ Vero in attached cultures. During adaptation a seeding density of 2×10^4 cells/cm² was used to obtain cultures that reached confluency within four days. Vero cells took 3 - 4 passages to adapt to EX-CELL™ Vero.

EX-CELL™ Vero supported HSV-2 production in Vero cells. The cells exhibited classic CPE in culture and produced HSV-2 titers in the range of $10^{6.0}$ TCID₅₀/mL. As shown in Figure 5, this range is comparable with those seen in serum-supplemented cultures.

References

1. Reed. L.J. and Muench, H.A., *A Simple Method of Estimating Fifty Per Cent Endpoints*, Am J Hyg. 1938;27:493-7.

Figure 5

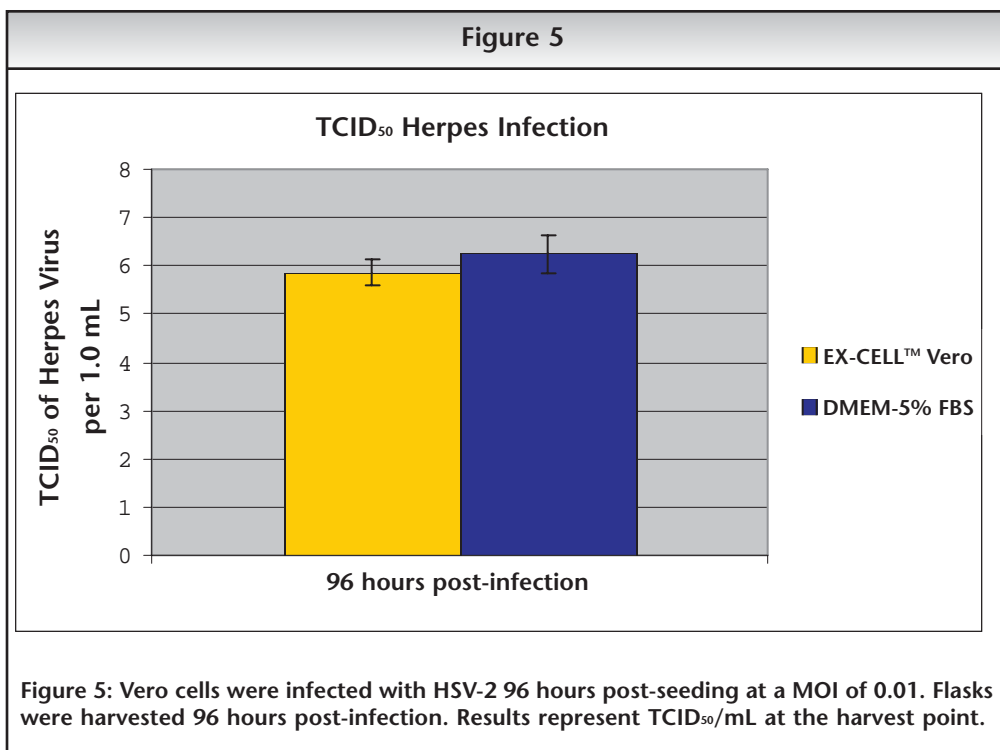


Figure 5: Vero cells were infected with HSV-2 96 hours post-seeding at a MOI of 0.01. Flasks were harvested 96 hours post-infection. Results represent TCID₅₀/mL at the harvest point.

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