

# Research Report

## PER.C6® Cell Growth and Adenovirus Production in EX-CELL™ VPRO Serum-Free Medium

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### Introduction

The PER.C6® cell line (Crucell N.V., Leiden, The Netherlands) is a fully characterized human cell line utilized in cell-based vaccine manufacturing and for production of recombinant adenoviral vectors and therapeutic monoclonal antibodies. We have designed and optimized EX-CELL™ VPRO Serum-Free Medium for Retinoblast Cells, a serum-free, animal-protein free medium for PER.C6® cells. EX-CELL™ VPRO is intended to facilitate large-scale, high-density suspension culture and to support recombinant adenovirus production. 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.

Growth studies in EX-CELL™ VPRO Medium were performed in roller bottles and evaluated PER.C6® cell densities, viabilities and Adenovirus Type 5 (Ad5) production. Average PER.C6® cell densities in roller bottles were between  $1.7\text{-}2.8 \times 10^6$  cells/mL with doubling times of approximately 30 hours. Ad5 production was in the range of  $2\text{-}5 \times 10^9$  TCID<sub>50</sub>/mL. We conclude that EX-CELL™ VPRO supports rapid PER.C6® cell growth in roller bottles and generates high density, highly viable cultures that are capable of adenovirus production.

### Materials

#### Cells

- PER.C6®, Crucell N.V. (Leiden, The Netherlands)

#### Virus

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

### Serum-Free Media

- EX-CELL™ VPRO Serum-Free Medium for Retinoblast Cells, SAFC Biosciences, Catalog No. 14560
- L-glutamine was aseptically added to EX-CELL™ VPRO 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 Biosciences, Catalog No. 59202
- Dulbecco's Modified Eagle's Medium (DMEM)/High Modified, SAFC Biosciences, Catalog No. 51444
- Fetal Bovine Serum (FBS) - Gamma Irradiated, SAFC Biosciences, Catalog No. 12107
- 0.25% Trypsin (1X) 0.1% EDTA Solution, SAFC Biosciences, Catalog No. 59229

### Methods

#### Growth Studies

PER.C6® cells were adapted to EX-CELL™ VPRO by direct adaptation from serum-free medium. Cells were subcultured at  $3 \times 10^5$  cells/mL directly into pre-warmed EX-CELL™ VPRO (100 mL volume per 490 cm<sup>2</sup> roller bottles). The initial carry-over of residual culture medium was less than 25%. Flasks were overlaid with 10% CO<sub>2</sub> (tightened caps) and incubated at 37 C on a roller rack at 1 rpm. Cultures were passaged twice per week (every 3 or 4 days) and were considered fully adapted after 6 passages. After adaptation, a working cell bank was frozen at  $1 \times 10^7$  cells/mL in a cryopreservation medium consisting of 45% conditioned medium, 45% fresh medium and 10% dimethyl sulfoxide (DMSO). Recovery of frozen PER.C6® cells and growth characteristics in EX-CELL™ VPRO were subsequently examined in roller bottles using the same methods described above.

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### Ad5 Viral Infection and TCID<sub>50</sub> Titration

Three independent infections with wild-type Ad5 were performed in EX-CELL™ VPRO. Cells were seeded on day 0 at  $5 \times 10^5$  cells/mL and infected on day 2 at approximately  $1 \times 10^6$  cells/mL with Ad5 at a multiplicity of infection (MOI) of 10. 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 µm sterile syringe filter and stored frozen (-70 C) until titered.

Titration were performed in 96-well microtiter plates as follows: PER.C6® cells were seeded in DMEM with 6 mM L-glutamine, 10 mM MgCl<sub>2</sub> (required for attachment) and 5% FBS at  $1 \times 10^4$  cells/100 mL/well. After cell attachment, the supernatant was removed and immediately replaced with viral dilutions. Duplicate serial dilutions ( $10^{-1}$  to  $10^{-11}$ ) of each lysate were made in DMEM with 2 mM L-glutamine, 10 mM MgCl<sub>2</sub> and 2% FBS and 200 µL was added to each well. Plates were incubated at 37 C, 10% CO<sub>2</sub> for 14 days, then observed on an inverted microscope for cytopathic effect (CPE). The titer (T) was determined as follows:

$$T = [10^{1+d(S-0.5)}] / 2$$

Where d = Log<sub>10</sub> of the dilution and S = Sum of the ratios

### Results

PER.C6® cells have been carried in multiple lots of EX-CELL™ VPRO for more than 20 passages (in-house studies). Average PER.C6® cell densities in roller bottles range between  $1.7\text{-}2.8 \times 10^6$  cells/mL (when subcultured every 3 - 4 days) with doubling times of approximately 30 hours. Figure 1 depicts the typical growth pattern of PER.C6® cells in EX-CELL™ VPRO in unfed roller bottle cultures over a period of 10 days. In these studies, EX-CELL™ VPRO supported exponential PER.C6® cell growth for 5 days with viabilities above 90%.

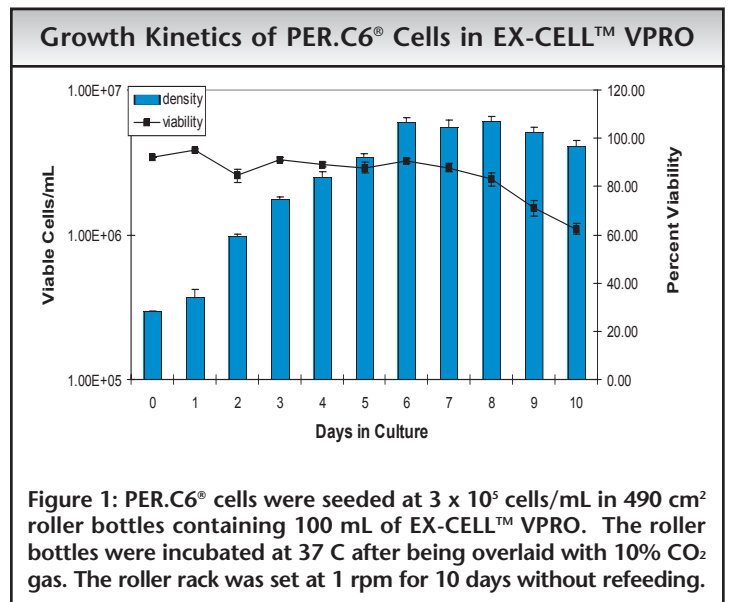


Figure 1: PER.C6® cells were seeded at  $3 \times 10^5$  cells/mL in 490 cm<sup>2</sup> roller bottles containing 100 mL of EX-CELL™ VPRO. The roller bottles were incubated at 37 C after being overlaid with 10% CO<sub>2</sub> gas. The roller rack was set at 1 rpm for 10 days without refeeding.

PER.C6® cells in EX-CELL™ VPRO also supported the production of Ad5 with yields in the range of  $2\text{-}5 \times 10^9$  TCID<sub>50</sub>/mL (Figure 2). Virus production was greatest between 48 - 96 hours post-infection. (We should note that this was a preliminary investigation and not an optimized study and that a pilot study is highly recommended to determine optimal parameters for your recombinant virus production.)

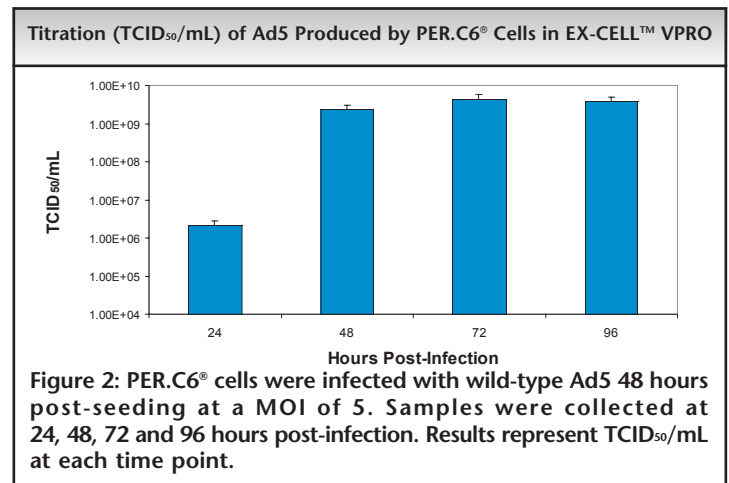


Figure 2: PER.C6® cells were infected with wild-type Ad5 48 hours post-seeding at a MOI of 5. Samples were collected at 24, 48, 72 and 96 hours post-infection. Results represent TCID<sub>50</sub>/mL at each time point.

### Conclusions

- EX-CELL™ VPRO produces high-density, highly viable PER.C6® cultures with up to  $6 \times 10^6$  viable cells/mL in roller bottles.
- EX-CELL™ VPRO supports highly viable PER.C6® cultures with most cultures above 95% viability.
- PER.C6® cells in EX-CELL™ VPRO produce high yields of adenovirus, up to  $5 \times 10^9$  TCID<sub>50</sub>/mL.
- EX-CELL™ VPRO is serum-free, animal-protein free, and contains very low levels of recombinant protein, facilitating downstream purification of products.



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