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Technical Bulletin

HeLa S3 Cell Growth in EX-CELL™ HeLa Serum-Free Medium

Introduction

The HeLa cell line, obtained more than 50 years ago, is still one of the most widely used cell lines around the world. The cell line (a cervical adenocarcinoma cell line with fibroblastic growth properties) has been extensively studied and is a remarkable research tool for understanding the intracellular signaling and gene regulation of cancerous and noncancerous cells and in the advancement of anticancer agents. A wide variety of viruses are also being studied with HeLa cell lines in an effort to develop pharmaceutical products targeted toward these viruses (e.g. Human Immunodeficiency Virus (HIV), Human Papilloma Virus (HPV), Adenovirus and Vaccinia Virus).

EX-CELL™ HeLa Serum-Free Medium for HeLa Cells is an animal-protein free, serum-free medium developed to support large-scale, high-density suspension culture and virus production in HeLa cells. EX-CELL™ HeLa is formulated without L-glutamine, avoiding problems associated with L-glutamine degradation and improving product shelf life. Our experiments with HeLa S3 cells (a clonal derivative capable of suspension growth) show EX-CELL™ HeLa supports high-density, serum-free HeLa cell growth and supports the production of adenovirus.

Materials

Cells

- HeLa S3 Cells, American Type Culture Collection (ATCC), ATCC No. CCL-2.2
- HEK 293 Cells, ATCC, ATCC No. CRL-1573
- Human adenovirus C deposited as Adenovirus type 5 (Ad5), ATCC, ATCC No. VR-5

Media and Supplements

- Minimum Essential Medium Alpha Modification (αMEM), SAFC Biosciences, Catalog No. 51451
- EX-CELL™ HeLa, SAFC Biosciences, Catalog No. 14590 (L-glutamine was added to EX-CELL™ HeLa at a final concentration of 6 mM at time of use)
- Dulbecco's Modified Eagle's Medium/High Modified (DMEM/High), SAFC Biosciences, Catalog No. 51441
- Fetal Bovine Serum (FBS) Gamma Irradiated, SAFC Biosciences, Catalog No. 12107
- L-Glutamine 200 mM Solution, 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
- Trypan Blue solution, Sigma-Aldrich Co., Catalog No. T8154

Methods

Direct Adaptation to EX-CELL™ HeLa:

HeLa S3 cells were started from frozen cells as adherent cultures in αMEM + 10% FBS, expanded in T-75 cm^2 flasks and then adapted to shakers (in αMEM + 10% FBS). Cells were then subcultured directly into EX-CELL™ HeLa at a seeding density of 3 x 10^5 cells/mL (30 mL of media/flask) without weaning. HeLa S3 cells continued to be subcultured every three days (72 hours ± 6 hours) at a seeding density of 3 x 10^5 cells/mL. The flasks (vented-cap 125 mL shaker flasks) were incubated on an orbital shaker at 120 - 150 rpm and were maintained at 37 C in a humidified incubator with 5% CO_2. Cell density and viability was determined by standard counting technique using a hemocytometer and trypan blue exclusion. All cultures were maintained using aseptic technique and without the use of antibiotics or fungicides. All experiments were performed using triplicate determinations, unless otherwise noted.

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Adenovirus Production and $TCID_{50}$ Titrations

Adenoviral propagation was initiated in HeLa S3 cells by adding the required amount of Ad5 viral stock, based on the multiplicity of infection (MOI) and cell density, in shaker cultures. Cultures were infected immediately post-seeding at 3×10^5 cells/mL with a MOI of 1. Cultures were incubated at 37 C, 5% CO_2 and shaken at 125 rpm. Samples were removed at 24-hour periods after infection and frozen at -70 C. The samples were lysed by freeze-thawing (-70 C/37 C) three times, then centrifuged (~ 2000 *g*) for 10 minutes to pellet the cellular debris. The supernatants were transferred to new tubes and either immediately titrated or frozen at -70 C until titration.

Titration was performed by Tissue Culture Infective Dose 50 ($TCID_{50}$/mL) using HEK 293 cells. HEK 293 cells in DMEM + 10% FBS were harvested by trypsinization, counted and diluted to a final concentration of 1×10^5 cells/mL in DMEM supplemented with 4% FBS and 4 mM L-glutamine. 1×10^4 cells/well were dispensed into 96-well microtiter plates and allowed to attach at 37 C.

Duplicate serial dilutions of each viral lysate were prepared in unsupplemented DMEM (1:10 dilutions; the 10^{-3} to 10^{-10} dilutions were plated). Each viral lysate was titrated in duplicate (i.e. on two plates) and the final titers were averaged to yield the final result. 100 µl of each dilution was dispensed in wells 1 - 10 (wells 11 and 12 in all rows served as controls). The plates were incubated at 37 C, 5% CO_2 for 10 days, then observed on an inverted microscope for CPE.

Results

Growth in EX-CELL™ HeLa:

HeLa S3 cultures were maintained in EX-CELL™ HeLa for more than 25 passes (Figure 1).

Figure 1

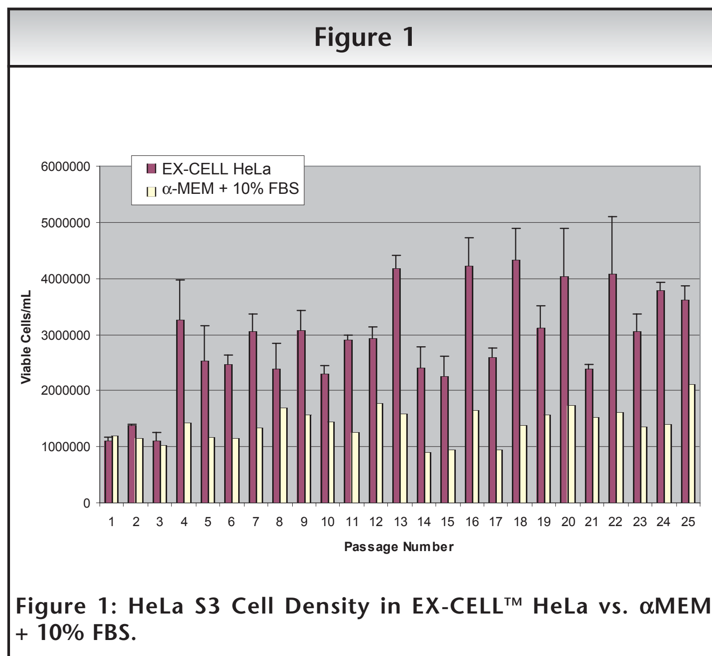

Figure 1: HeLa S3 Cell Density in EX-CELL™ HeLa vs. αMEM + 10% FBS.

Cell density in EX-CELL™ HeLa averaged ~ 2.9×10^6 cells/mL and the average doubling time was ~ 24.1 hours. The average density in αMEM + 10% FBS was about 1.4×10^6 cells/mL and the average doubling time was approximately 35 hours (n=1 flask per passage for αMEM + 10%). Table 1 illustrates the ranges seen in cell density, viability and doubling time over the course of the entire experiment.

Table 1. HeLa S3 Growth Characteristics in EX-CELL™ HeLa (Direct Adaptation)

	Viable Cells/mL (x 10^6)	Percent Viable	Doubling Time (Hrs)
Average	2.9	96.9	24.1
Standard Deviation	1.1	2.9	6.5
Range (Min - Max)	0.9 - 6.1	82.4 - 100.00	16.5 - 48.3

HeLa S3 cell counts during the first three passes in EX-CELL™ HeLa mimicked those seen in αMEM + 10% FBS. Beginning with the fourth passage, the cell counts in EX-CELL™ HeLa consistently doubled or tripled those in serum during the course of the study (Figure 1). During, and after adaptation the cells in EX-CELL™ HeLa appeared very healthy. Cultures were mostly unicellular, however clumps of up to 30 - 40 cells were noted and became more prevalent as culture density increased (> 3 - 4 x 10^6 cells/mL).

Adenovirus Production

Adenovirus production in EX-CELL™ HeLa was examined using a variety of MOI's, seeding densities and temperatures. The most favorable results were obtained when HeLa cells were infected at an MOI of 1, immediately after seeding at 3 x 10^5 cells/mL (Figure 2). Viral titers of approximately 1.5 x 10^9 $TCID_{50}$/mL were obtained in EX-CELL™ HeLa. These results were within one-half log value of those seen in αMEM + 10% FBS.

Figure 2

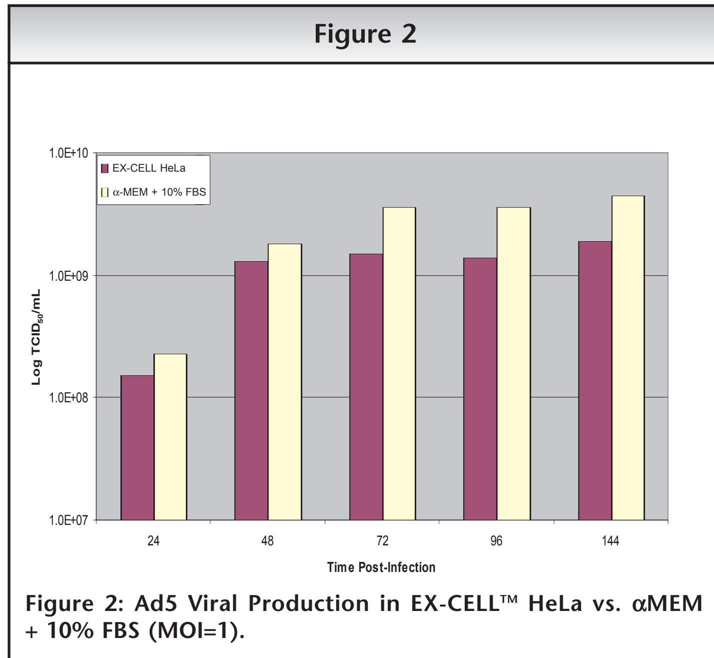

Figure 2: Ad5 Viral Production in EX-CELL™ HeLa vs. αMEM + 10% FBS (MOI=1).

Summary

- EX-CELL™ HeLa is a serum-free medium for HeLa suspension cultures.
- EX-CELL™ HeLa is formulated without L-glutamine, thereby increasing product stability and shelf life.
- EX-CELL™ HeLa contains low levels of recombinant protein, facilitating purification and processing of products.
- HeLa S3 cells easily adapt to suspension culture in EX-CELL™ HeLa with very little cellular aggregation.
- EX-CELL™ HeLa produces high-density, highly viable HeLa S3 cultures with up to 4 x 10^6 viable cells/mL in shaker flasks.
- EX-CELL™ HeLa supports highly viable HeLa S3 cultures with cultures above 96% viability.
- HeLa S3 cells grown in EX-CELL™ HeLa produce adenovirus with titers similar to those seen in αMEM + 10% FBS.

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