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Technical Bulletin

Suspension MDCK Cell Culture in EX-CELL™ MDCK Serum-Free Medium

Introduction

EX-CELL™ MDCK is a serum-free, animal-protein free medium developed for the Madin Darby Canine Kidney (MDCK) cell line. Previous studies[1] have shown the utility of EX-CELL™ MDCK for growing high-density MDCK adherent cultures for the production of canine adenovirus. The current studies illustrate that adherent MDCK cells (adapted to EX-CELL™ MDCK medium) can simply be transferred into shaker flasks and grow well in suspension, with little adaptation. Doubling times in MDCK suspension cultures are similar to those seen in adherent cultures (30 - 40 hours) and the cells grow in single-cell suspension. Maximum MDCK cell densities in suspension cultures are approximately 5 x 10^6 cells/mL with viabilities >90%. Suspension cultures remain susceptible to viral infection, and in this study, Canine Adenovirus (CAV) titers in excess of 10^9 infectious particles/mL were obtained.

Materials

Cells and Virus

- Madin Darby Canine Kidney (MDCK), American Type Culture Collection, ATCC No. CCL-34
- Canine Adenovirus (CAV), Strain: Toronto A 26/61, American Type Culture Collection, ATCC No. VR-800

Media and Supplements

- EX-CELL™ MDCK Serum-Free Medium for MDCK Cells, SAFC Biosciences, Catalog No. 14581
- Dulbecco's Phosphate Buffered Saline (DPBS Modified), SAFC Biosciences, Catalog No. 59321
- 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
- Trypsin inhibitor from Glycine max (soybean) (STI), Sigma-Aldrich, Catalog No. T6522

Adaptation to Suspension Culture

MDCK cells were initiated as adherent cultures from a frozen working cell bank of cells previously adapted to EX-CELL™ MDCK medium. The cells were passaged several times in T-flasks to expand the quantity of cells. T-flasks were trypsinized using standard techniques[1,2] and STI was utilized to inhibit the trypsin. The cells were transferred to centrifuge tubes and centrifuged at 1000 rpm (200 *g*) to remove the trypsin and inhibitor. The cells were resuspended in fresh EX-CELL™ MDCK medium (supplemented with 6 mM L-glutamine) and cell densities and viabilities were determined by trypan blue exclusion using a Cedex cell counter (Innovatis AG, Bielefeld, Germany). The cells were seeded in 125 mL shaker flasks at 5 x 10^5 cells/mL in a total volume of 30 mL. All flasks were placed on an orbital shaker (~120 rpm) in a 37 C humidified incubator with 5% CO_2. MDCK suspension cultures were subcultured every 3 - 4 days, and were monitored over eight passages; a growth curve was also generated over nine days in unfed cultures. All determinations were performed in triplicate.

CAV Infection of MDCK Suspension Cultures

MDCK cells were seeded at 3 x 10^5 cells/mL in EX-CELL™ MDCK medium and incubated as described in the previous section. The following day (24 hours post-seeding), cultures were infected with CAV at MOI's (Multiplicities of Infection) of 0.1 and 0.01 and returned to the incubator. Daily samples were obtained for five days and frozen (-70 C) until titration. Titration was performed by $TCID_{50}$ on adherent MDCK cells in EX-CELL™ MDCK medium.

Results

Adaptation and Growth in Suspension

In these studies, adherent MDCK cells readily adapted to growth in suspension in EX-CELL™ MDCK medium. The first passage in suspension shows a 2 - 3 fold increase in doubling time versus the adherent control (Figure 1). However, this

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increase was only apparent during the first suspension passage and doubling times in subsequent passages were equivalent to adherent cultures (approximately 30 - 40 hours). Cell densities in suspension ranged from approximately 1-3 x 10^6 cells/mL, with culture viabilities in excess of 93%, when subcultured every 3 - 4 days (Figure 2). The growth curve (Figure 3) indicates that maximal cell densities nearing 5 x 10^6 cell/mL were obtained on days 7 - 8, after which the culture viability and density rapidly declined.

CAV Infection

EX-CELL™ MDCK supported CAV production in suspension MDCK cells. There was no significant difference in virus production between the two MOI's tested. Both infection conditions produced peak $TCID_{50}$ titers in the range of 10^8 - 10^9 particles/mL between 72 - 96 hours post-infection (Figure 4).

Summary

EX-CELL™ MDCK is a serum-free medium developed for adherent MDCK cell culture. A previous report[1] illustrated the adaptation, growth and virus production characteristics of adherent MDCK cells in EX-CELL™ MDCK medium. The current study sought to determine if MDCK cells could be adapted to grow in suspension culture in EX-CELL™ MDCK and determine whether or not the cells would be susceptible to virus infection.

Adaptation of adherent MDCK cells to suspension was easily accomplished without a lengthy procedure; cells were simply transferred to shaker flasks and routinely subcultured. Although doubling times were long during the first suspension passage, doubling times quickly returned to normal and culture viability was above 90% throughout the study. Infection of the suspension MDCK cultures with CAV yielded titers between 10^8 - 10^9 $TCID_{50}$/mL, proving that the suspension cultures were capable of producing virus. We conclude that EX-CELL™ MDCK medium is suitable for both adherent and suspension MDCK cell lines.

References

[1]Evaluation of MDCK Cell Growth and Virus Production in EX-CELL™ MDCK, SAFC Biosciences, Literature No. R024.
[2]EX-CELL™ MDCK Serum-Free Medium for MDCK Cells, Product Information Sheet, SAFC Biosciences, Literature No. P14581.

Figure 1

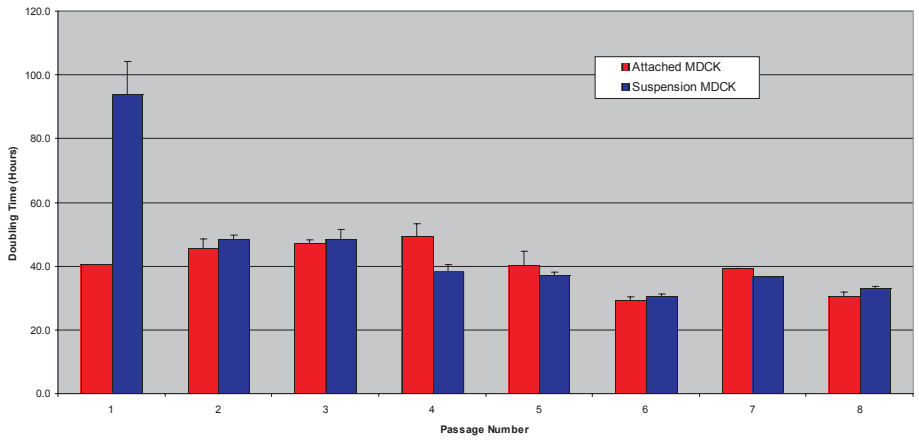

Figure 1: Doubling times of MDCK cells as attachment and suspension cultures in EX-CELL™ MDCK.

Figure 2

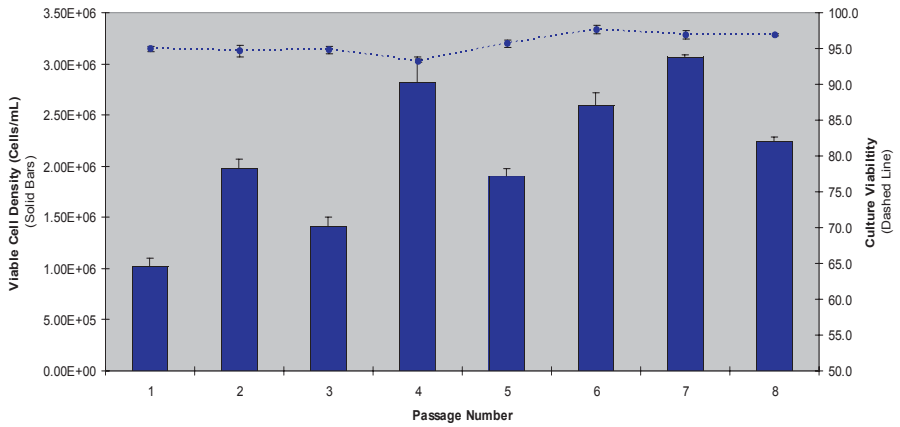

Figure 2: Adaptation of MDCK cells to suspension culture in EX-CELL™ MDCK.

Figure 3

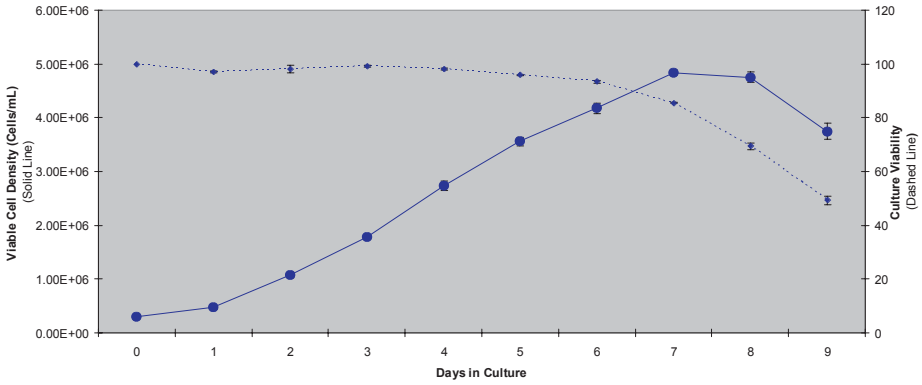

Figure 3: Growth curve of MDCK cells in suspension culture in EX-CELL™ MDCK.

Figure 4

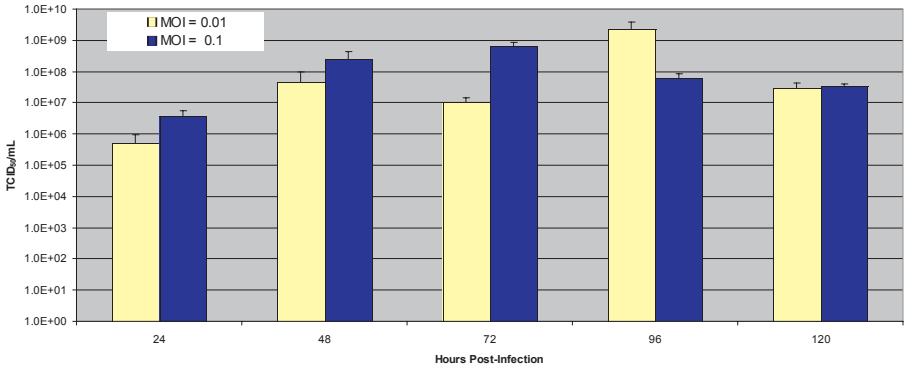

Figure 4: CAV production by suspension MDCK cells in EX-CELL™ MDCK.

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