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Technical Bulletin

Growth Characteristics of the *expres*SF+® Serum-Free Insect Cell Line in EX-CELL™ 420 Serum-Free Media

Introduction

The Baculovirus Expression Vector System (BEVS) is a widely used tool for the production and expression of baculoviruses and recombinant proteins. The Sf9 and Sf21 insect cell lines, derived from the ovaries of the fall armyworm *Spodoptera frugiperda*, have been the cell lines of choice when using the BEVS technology. Recently, Protein Sciences Corporation[1] developed a new cell line derived from *Spodoptera frugiperda*, which is morphologically and genetically different from the Sf9 cell line[2]. The cell line, *expres*SF+®, was developed using a series of stringent selection steps in serum-free medium with added insulin[2]. The cell line was designed to possess characteristics that favor the commercial production of baculoviruses and recombinant proteins.

EX-CELL™ 420 Serum-Free Medium for Insect Cells is a serum-free, protein-free medium designed and optimized for suspension culture of Spodopteran cell lines. Previous studies with this medium have shown that Sf9 and Sf21 cells adapt easily to EX-CELL™ 420, and that EX-CELL™ 420 supports high cell densities, baculovirus production and secreted and intracellular recombinant protein expression[3,4]. Experiments were designed to assess the ease with which *expres*SF+® cells adapt to EX-CELL™ 420, to evaluate cell growth and viability in shaker flask culture and to determine freezing conditions. The results demonstrate that *expres*SF+® cells quickly adapt to EX-CELL™ 420, with no observed decrease in cell growth or viability. *expres*SF+® cell viabilities and densities in EX-CELL™ 420 were comparable to those seen in cultures grown in Sf-900 II SFM, (Invitrogen Corporation) and *expres*SF+® cells frozen in fresh EX-CELL™ 420 medium with 10% dimethyl sulfoxide (DMSO) exhibited viabilities of about 90% upon thawing from storage in liquid nitrogen.

Materials

Cells

- *expres*SF+® Serum-Free Insect Cells, Protein Sciences Corporation, Catalog No. 1000

Media and Supplements

- Sf-900 II SFM, Invitrogen Corporation, Catalog No. 10902
- EX-CELL™ 420, SAFC Biosciences, Inc., Catalog No. 14420
- L-Glutamine Solution 200 mM, SAFC Biosciences, Inc., Catalog No. 59202
- DMSO, Sigma-Aldrich Co., Catalog No. D-2650

Methods

*expres*SF+® Culture Initiation

*expres*SF+® cells were handled and subcultured per the manual (Version 1.4) provided by the manufacturer. As such, a new vial of frozen *expres*SF+® cells was thawed rapidly with agitation in a 28 C water bath. Once a small ice pellet remained, the contents of the vial were added to 100 mL pre-warmed Sf-900 II SFM in a 250 mL spinner flask. The flask was incubated at 28 C in a non-CO_2 atmosphere, with constant stirring at 100 rpm. The cells were subsequently subcultured into triplicate shaker flasks (50 mL volume per 125 mL flask) at a seeding density of 1.5 x 10^6 cells/mL. Flasks were incubated at 28 C, on an orbital shaker at 135 rpm. Cell counts and viability were monitored using trypan blue exclusion methods. Flasks were subcultured every 2 - 3 days using the same seeding density as above.

Direct Adaptation and Continuous Growth of *expres*SF+® Cells in EX-CELL™ 420

Cultures initiated in Sf-900 II SFM were passed five times to ensure the cultures were well established. On the sixth pass, the cells were directly subcultured into 100% EX-CELL™ 420 medium at a seeding density of 1.5 x 10^6 cells/mL. These cultures were monitored for an additional five passes to assess cell density and viability in EX-CELL™ 420. On the sixth pass, a kinetic growth curve was generated by obtaining daily cell counts, until culture viability dropped below 50%.

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Freeze/Thaw Evaluation of *expres*SF+® Cells in EX-CELL™ 420

To evaluate freezing conditions in EX-CELL™ 420, a cell bank of *expres*SF+® cells was prepared. Mid-log phase cells (> 95% viable) were harvested and centrifuged to remove spent medium. The cells were resuspended at 3 x 10^7 cells/mL in a cryopreservation medium consisting of 90% fresh EX-CELL™ 420 medium and 10% DMSO. Cells were frozen at a controlled rate and stored under liquid nitrogen. Cell viability post-thaw was determined by thawing 3 vials (1 mL each) of cells and combining them directly into a 125 mL shaker flask containing 50 mL room temperature media (triplicate determinations were performed). The flasks were shaken (135 rpm) for 24 hours at 28 C, at which time cell density and viability were determined.

Results

Direct Adaptation and Continuous Growth of *expres*SF+® Cells in EX-CELL™ 420

*expres*SF+® shaker cultures adapted easily from Sf-900 II SFM to EX-CELL™ 420 without any noticeable lag in cell growth or decrease in cell viability (see Figure 1). The cells appeared healthy and there was no clumping of cells even at higher densities. Cell densities in EX-CELL™ 420 were typically around 6 x 10^6 cells/mL (range 3.7 x 10^6 to 9.9 x 10^6 cells/mL) and displayed an average doubling time of 26 hours. Culture viabilities ranged from 94.4% to 99.5%, with most determinations above 96%.

Figure 1

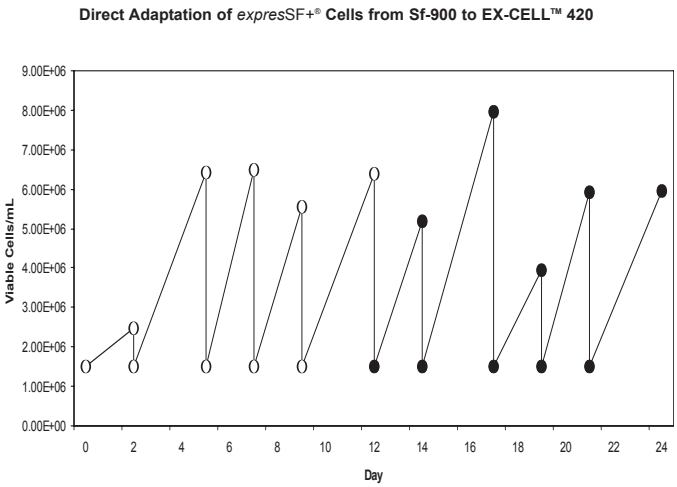

Figure 1: *expres*SF+® cells growing in Sf-900 II SFM (open circles) were directly subcultured into EX-CELL™ 420 (shaded circles) at the sixth passage (Day 12).

To demonstrate the kinetic growth curve of *expres*SF+® cells in continuous culture, *expres*SF+® cells were maintained in EX-CELL™ 420 for seven days (see Figure 2). Cell densities increased exponentially over the first two days then appeared to plateau between days two to four, reaching maximum densities of approximately 7 x 10^6 cells/mL. Culture viabilities remained high (above 95%) until day four, after which viabilities dropped to below 50% by day seven. Based on these results, it was confirmed that the optimal time for subculture is during the exponential growth phase of the cells, which is between two to three days in culture.

Figure 2

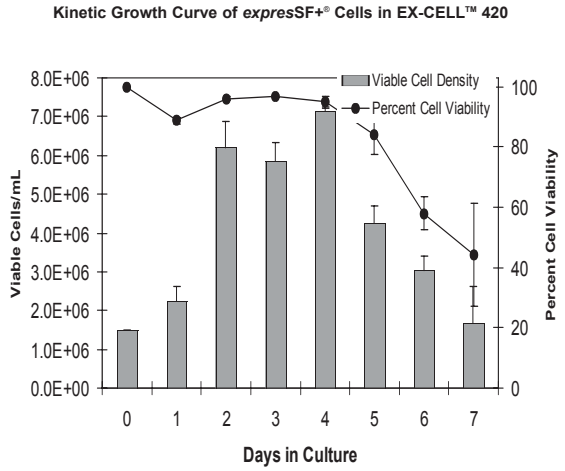

Figure 2: *expres*SF+® cells were grown in EX-CELL™ 420 for seven days without refeeding.

Freeze/Thaw Evaluation of *expres*SF+® Cells in EX-CELL™ 420

Table 1 indicates the density and viability of cells frozen in fresh EX-CELL™ 420 with 10% DMSO at 24 hours post-thaw. These results indicate that *expres*SF+® cells can be frozen and easily recovered in EX-CELL™ 420.

Table 1. Density and Viability of *expres*SF+® Cells 24 Hours Post-Thaw

	Cell Density (cells/mL)	Cell Viability (%)
Flask 1	2.32 x 10^6	92.1
Flask 2	2.76 x 10^6	86.3
Flask 3	3.12 x 10^6	91.8

Conclusions

- *expres*SF+® cells can be transferred directly into EX-CELL™ 420 from Sf-900 II SFM without an adaptation period.
- *expres*SF+® cells grown in EX-CELL™ 420 achieve densities of 6-7 x 10^6 cells/mL with viabilities greater than 95%.
- *expres*SF+® cells can be frozen and recovered in EX-CELL™ 420 with only the addition of 10% DMSO.

References

1. Protein Sciences Corporation, Meriden, CT

2. *expres*SF+® Serum-Free Insect Cell Line, Manual Version 1.4, Protein Sciences Corporation

3. Dianne E. Potts, Justine A. Malinski, Laura T. Kakach and Sarah L. Gilliland, Commercially Available Serum-Free Insect Media: A Comparison of Sf9 Growth Dynamics and Protein Production, SAFC Biosciences Literature Reference R011, 2000.

4. Susan E. Lenk, Thomas W. Irish and Karen J. Etchberger, EX-CELL™ 420 Serum-Free Medium for the Growth of Spodopteran (Sf9 and Sf21) Insect Cells, SAFC Biosciences Literature Reference R015, 2000.

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