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Research Report

EX-CELL® 420 Serum Medium for the Growth of Spodopteran (Sf9 and Sf21) Insect Cells

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Abstract

Insect cells are commonly used for the expression of recombinant proteins and virus production in a variety of applications including vaccines, diagnostics and biopesticides. Cells derived from the fall armyworm, *Spodoptera frugiperda,* have been the cells of choice for baculovirus infection and protein production. EX-CELL 420 Serum-Free Medium for Insect Cells was developed and optimized to support cell growth, baculovirus production and recombinant protein expression by the Baculovirus Expression Vector System (BEVS) in Sf9 and Sf21 cells. Cells can be transferred directly from adherent cultures grown in Hink's TNM-FH supplemented with 10% serum into EX-CELL 420 suspension culture without an adaptation period.

Sf9 cells cultured in EX-CELL 420 routinely achieve cell densities of $>10^7$ cells/mL with viabilities greater than 95% and can be maintained for more than 10 days at these densities. Cultures seeded at low density (2.5×10^5 cells/mL) over multiple passages do not exhibit the typical 24 - 48 hour lag phase seen with many serum-free media. Both Sf9 and Sf21 cultures have been maintained in EX-CELL 420 for more than 20 passages. Sf9 cells grown in EX-CELL 420 were infected with a pVL941 CAT clone HTS at an MOI of 5 and the resulting viral titer was 0.6×10^8 pfu/mL. Expression of IL-6, CAT and ß-galactosidase by Sf9 cells grown in EX-CELL 420 is comparable to that obtained from cells grown in a serum containing medium.

Introduction

Insect cells are commonly used for the expression of recombinant proteins and virus production in a variety of applications including basic research, vaccines, diagnostics and biopesticides. For many years, Sf9 and Sf21 cells, derived from the fall armyworm, *Spodoptera frugiperda*, have been the cells of choice for baculovirus production (both wild-type and recombinant) and recombinant protein expression by the Baculovirus Expression Vector System (BEVS) [1-3].

Several media have been developed for the growth of insect cells including Grace's, TNM-FH, IPL-41, TC-100, Mitsuhashi and Maramorosch [4-8]. Each of these formulations requires supplementation with 5 - 20% Fetal Bovine Serum (FBS) to provide additional nutrients and attachment factors. However, the cost of FBS, regulatory concerns and difficulties with downstream processing make serum less attractive for large-scale insect cell cultures. The need for more cost-effective, defined formulations has resulted in the development of several low-serum and serum-free media for insect cell culture [9-15].

EX-CELL 420 is a complete medium developed and optimized for the serum-free growth of Spodopteran cell lines as either adherent or suspension cultures. This medium is protein-free when measured by standard methods. Cells can be transferred directly from adherent cultures grown in serum-free or serum-containing media into EX-CELL 420 suspension cultures without weaning or adaptation. Sf9 cells cultured in EX-CELL 420 routinely achieve cell densities $> 10^7$ cells/mL with viabilities greater than 95% and can be maintained for more than 10 days at these densities. Cultures seeded at low density, (2.5×10^5 cells/mL), over multiple passages, do not exhibit the typical 24 - 48 hour lag phase common to many serum-free media. Sf9 and Sf21 cultures have been carried in EX-CELL 420 for more than 20 serum-free passages and successfully frozen without serum supplementation. Viral production and the expression of 3 proteins — ß-galactosidase, chloramphenicol acetyltransferase (CAT) and interleukin-6 (IL-6) — by Sf9 cells grown in EX-CELL 420 is comparable to that obtained from cells grown in Grace's + 10% FBS.

Materials and Methods

Cell Lines

- Sf9 cells (CRL1711) were obtained from the American Type Culture Collection (ATCC) (Rockville, MD).
- Sf21 cells (B821-01) were obtained from Invitrogen Corporation (Carlsbad, CA).

Cell Culture

- All cell culture reagents were supplied by SAFC (Lenexa, KS).
- Sf9 cells were transferred directly to EX-CELL 420 from ATCC frozen stocks as attachment cultures.
- Adaptation of Sf9 cells to serum-free control medium was accomplished by weaning from attachment cultures adapted to Hink's TNM-FH + 10% FBS.

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- Sf21 cells were transferred directly to EX-CELL® 420 from attachment cultures adapted to EX-CELL 400 Serum-Free Medium.
- Adaptation of Sf21 cells to serum-free control medium was accomplished by weaning from attachment cultures adapted to Hink's TNM-FH + 10% FBS.
- Cells in both serum-free media were adapted to suspension culture in 125 mL shaker flasks (Corning) with 50 mL media volume at 130 rpm.
- For growth curves, cells were seeded in duplicate flasks at either 2.5 x 10^5 cells/mL or 4.0 x 10^5 cells/mL in 50 mL volume of media. Daily cell counts and viabilities were determined by Trypan Blue exclusion. Cells were not refed during the cellular growth study.

Virus Production

- All virus production was carried out by the Invitrogen Custom Baculovirus Service (Carlsbad, CA).
- Sf9 cells grown in EX-CELL 420 and Grace's Insect Medium + 10% FBS were infected with a pVL941 CAT clone HTS at an MOI of 5.
- Viral titers were determined by plaque assays performed on the culture supenatants.

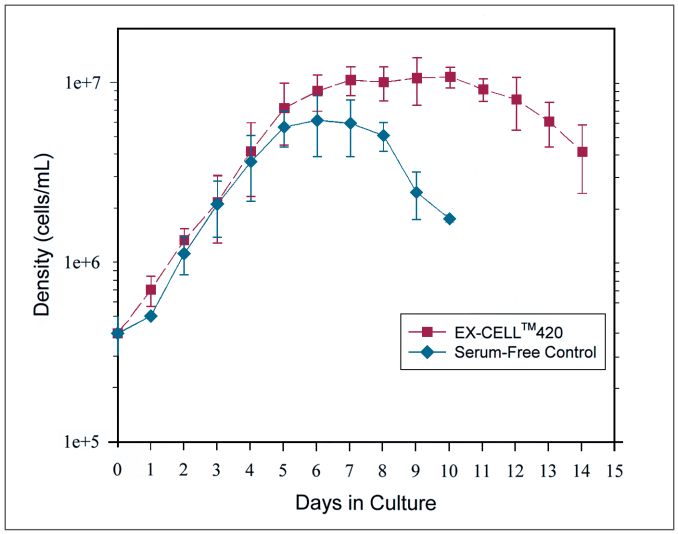
Protein Expression

- All protein expression was carried out by the Invitrogen Custom Baculovirus Service (Carlsbad, CA).
- ß-galactosidase, chloramphenicol acetyltransferase (CAT) and interleukin-6 (IL-6) were expressed in Sf9 cells grown in EX-CELL 420 and control cells grown in Grace's insect medium supplemented with 10% FBS. Cultures (50 mL) were infected with the respective recombinant baculovirus and then harvested at 72 hours post-infection.
- ß-galactosidase production was determined by specto-photometric activity assays. ß-galactosidase activity was normalized to total cell protein as determined by Bradford assay.
- CAT and IL-6 production were determined by western blot analysis using an HRP-conjugated anti-CAT antibody and a rat anti-human IL-6 antibody.

Conclusions

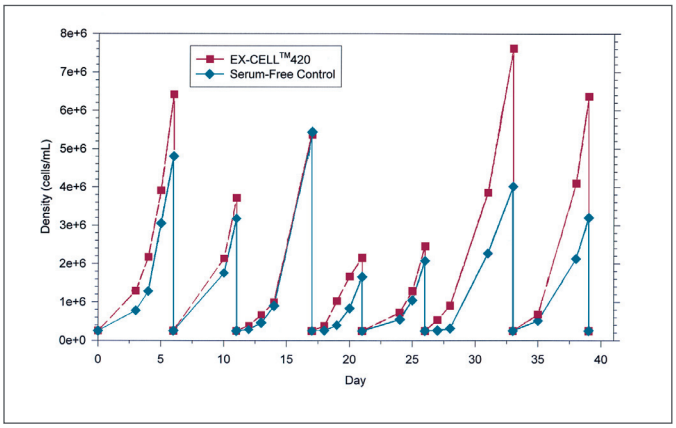
- Sf21 cells routinely achieve cell densities of > 7 x10^6 cells/mL with viabilities of > 95%.
- Virus production and protein expression in EX-CELL 420 is comparable to that obtained in serum-containing media.
- Cells can be transferred directly to EX-CELL 420 from a serum containing medium without an adaptation period.
- Sf9 cells grown in EX-CELL 420 routinely achieve cell densities of > 10^7 cells/mL with viabilities of > 95%.
- Sf9 cultures can be maintained for more than 10 days with no loss of viability.
- Sf9 cultures seeded at low density (2.5 x 10^5 cells/mL) do not exhibit the typical 24 - 48 hour lag phase seen with many serum-free media.

Figure 1. Growth of Sf9 Cells in EX-CELL 420 and Serum-Free Control



Typical growth curves obtained for Sf9 cells from unfed suspension (shaker) cultures grown in EX-CELL 420 and a Serum-Free Control Medium. Cultures were seeded at 4 x 10^5 cells/mL in 125 shakers (50 mL media volume) and counted daily.

Figure 2. Growth of Sf9 Cells in EX-CELL 420 and Serum-Free Control



A graphic representation of the adaptation of Sf9 cells to EX-CELL 420 and a Serum-Free Control Medium in suspension (125 shakers with 50 mL media volume). Cultures were seeded at 2.5 x 10^5 cells/mL and counted daily.

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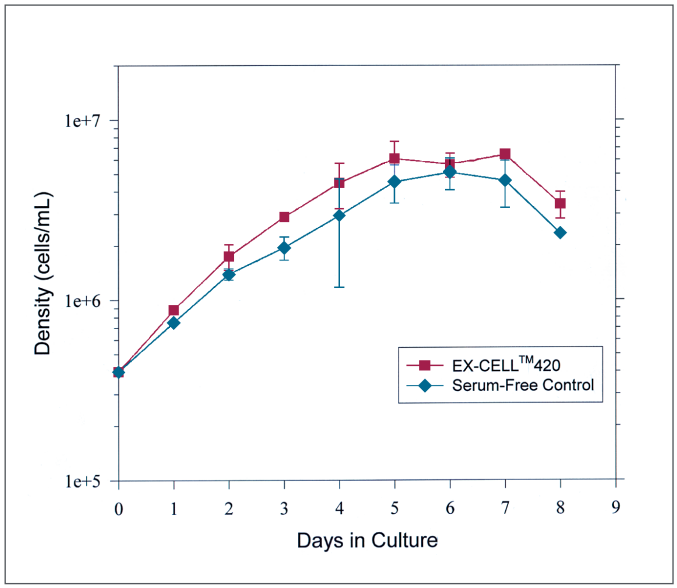
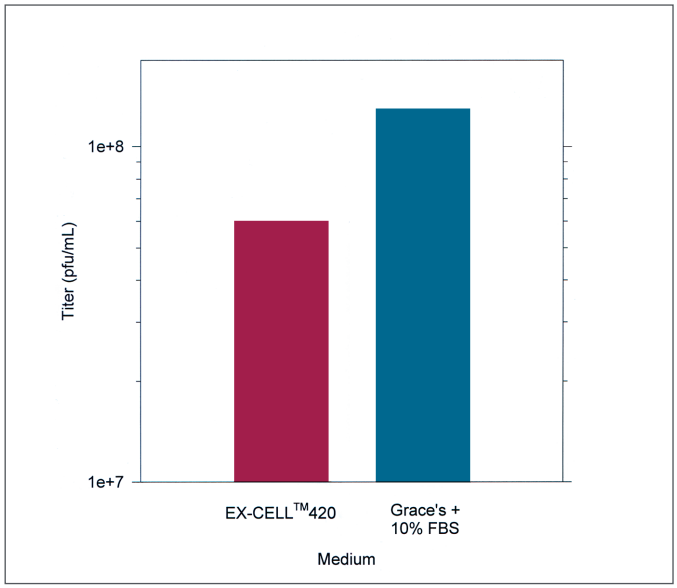
Figure 3. Growth of Sf21 Cells in EX-CELL® 420 and Serum-Free Control



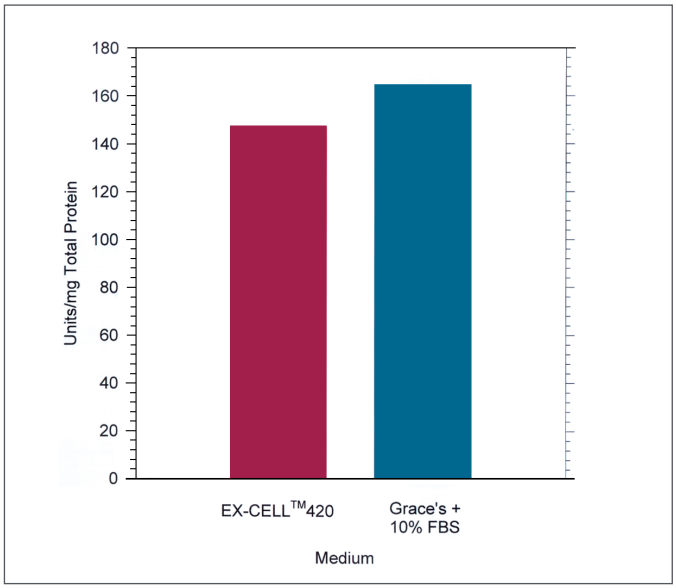
Typical growth curves obtained for Sf21 cells from unfed suspension (shaker) cultures grown in EX-CELL 420 and a Serum-Free Control Medium. Cultures were seeded at 4 x 10^5 cells/mL in 125 shakers (50 mL media volume) and counted daily.

Figure 4. Virus Production by Sf9 Cells in EX-CELL 420 (MOI = 5)



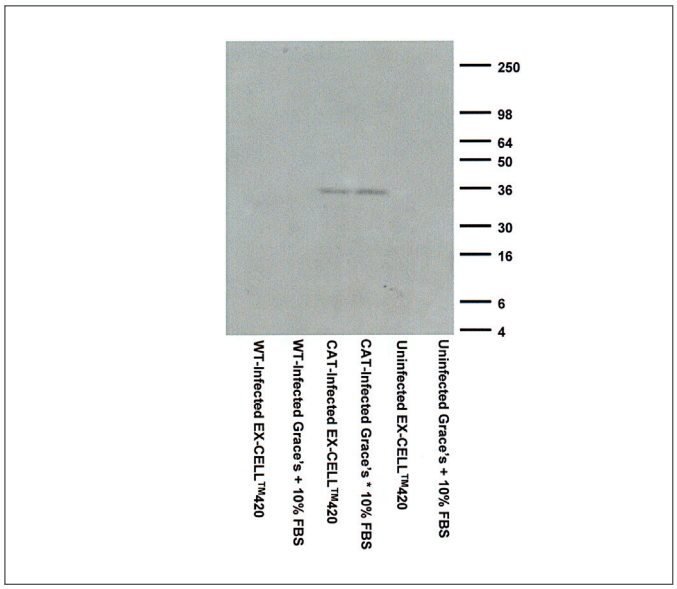
Sf9 cells were infected with a pVL941 CAT clone HTS at an MOI of 5. Cells were grown in both EX-CELL 420 and the control medium, Grace's + 10% FBS. The viral titer of the EX-CELL 420 cells is 0.6 x 10^8 pfu/mL and the control titer is 1.3 x 10^8 pfu/mL, a difference of 0.5 log.

Figure 5. ß-galactosidase Expression by Sf9 Cells in EX-CELL 420



Cells were grown in both EX-CELL 420 and the control medium, Grace's + 10% FBS. Cells were infected with a ß-galactosidase recombinant baculovirus high titer stock. Activity, as determined by spectrophotometric assay, is normalized to total cell protein.

Figure 6. CAT Expression by Sf9 Cells in EX-CELL 420



An autoradiograph of a western blot of cell lysates from CAT-recombinant virus infected Sf9 cells grown in EX-CELL 420 and the control medium, Grace's + 10% FBS. Additional controls include lysates from uninfected cells and lysates from cells infected with a wild type (wt) virus.

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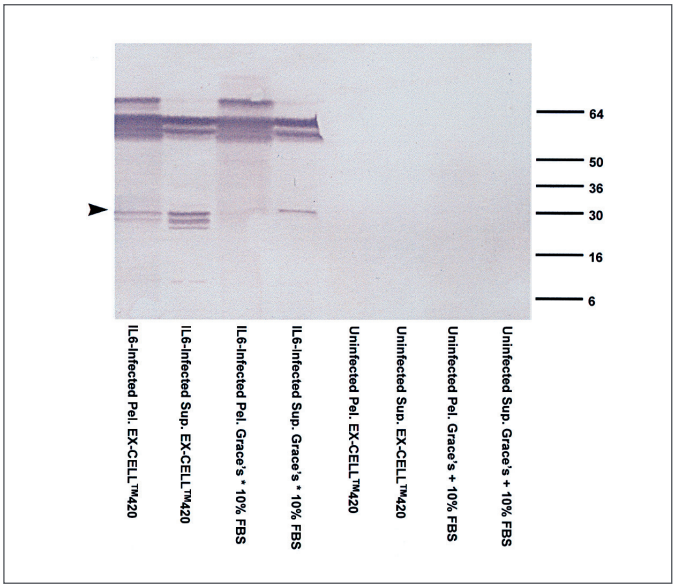
Figure 7. Interleukin-6 Expression by Sf9 Cells in EX-CELL® 420



A western blot of cell pellets (pel.) and supernatants (sup.) from IL-6 recombinant virus infected Sf9 cells grown in EX-CELL 420 and the control medium, Grace's + 10% FBS. The arrowhead indicates IL-6.

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