

Research Report

A Blind Comparison of Serum-Free Media Specifically Designed for the BTI-TN-5B1-4 (High Five™) Cell Line

Study independently performed by ATG Laboratories, Inc.,
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Abstract

In this blind study, four commercially available serum-free media, specifically designed for the suspension culture of the BTI-TN-5B1-4 (Tn-5, High Five™) cell line were compared. Suspension cultures of High Five™ cells were sequentially adapted to each of the four media. Three multiple-passage growth studies were performed with cell density and viability determined daily in each medium. Cell densities were found to be approximately equivalent in each medium, ranging between 2.3-4.6 x 10⁶ cells/mL. Cell viability was consistently above 98% in all media. Cultures were subsequently infected with a recombinant baculovirus expressing β-galactosidase and enzymatic activity was analyzed at 24, 48, 72 and 96 hours post-infection. β-galactosidase was expressed in each culture at every time point, with maximal expression at 48 hours post-infection. Cells cultured in EX-CELL™ 405 produced higher (p < 0.05) levels of recombinant β-galactosidase at both 48 and 72 hours post-infection than cells cultured in the other test media.

Introduction

Due to its high productivity, the *Trichoplusia ni* (cabbage looper) BTI-TN-5B1-4 (Tn-5, High Five™) cell line has become an attractive choice for recombinant protein production using the Baculovirus Expression Vector System (BEVS). Once thought to be an attachment-dependent cell line, High Five™ cells are now routinely grown in serum-free suspension culture allowing for large-scale recombinant protein production, with simplified purification. Many factors however, can influence the levels of protein expression when using the BEVS including: characteristics of the cell line, the protein being expressed, the viral construct and even the media used to culture the cells.

In an attempt to evaluate commercially available media specifically designed for the High Five™ cell line, four comparable serum-free media were examined under controlled conditions. The media evaluated in this study were HyQ® SFX-Insect™ (HyClone Laboratories, Inc.), EX-CELL™ 405 (SAFC Biosciences, Inc.), Express Five® SFM (Life Technologies, Inc.) and Ultimate Insect™ Serum-Free Medium (Invitrogen Corporation). All media

were purchased by SAFC Biosciences, Inc., repackaged, blind-labeled and then evaluated by an outside testing facility for ease of adaptation, growth characteristics and recombinant protein expression. All media were handled identically during the course of the studies and at no time was the outside laboratory aware of which media was being tested.

Materials

Cells

- High Five™ Cells (*Trichoplusia ni* BTI-TN-5B1-4) Invitrogen Corporation, Catalog No. B855-02

Serum-Free Media

- EX-CELL™ 405, SAFC Biosciences, Inc., Catalog No. 14405-1000M
- HyQ® SFX-Insect™, HyClone Laboratories, Inc., Catalog No. SH30278.02
- Express Five® SFM, Life Technologies, Inc., Catalog No. 10486-025
- Ultimate Insect Serum-Free Medium, Invitrogen Corporation, Catalog No. B101-01

Other Media and Supplements

- Grace's Insect Media, Life Technologies, Inc., Catalog No. 11605-094
- Fetal Bovine Serum (FBS), Life Technologies, Inc., Catalog No. 10099-141, Heat inactivated 56 C, 30 minutes
- Pluronic® F68, SAFC Biosciences, Inc., Catalog No. 59915-100M
- 200 mM L-glutamine, SAFC Biosciences, Inc., Catalog No. 59202-100M
- 10X Phosphate Buffered Saline, Life Technologies, Inc., Catalog No. 70011-044

Protein Expression Components

- Recombinant baculovirus BacPak6 Virus Stock, Clontech Laboratories, Inc., Catalog No. K1601-C
- β-Gal Assay Kit, Invitrogen Corporation, Catalog No. K1455-01

Methods

Serum-Free Media Preparation

All serum-free media were purchased from the respective manufacturers. No L-glutamine, other supplements (e.g. Pluronic® F68) or antibiotics were added to the media with the exception of the Express Five® SFM.

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L-glutamine was added to Express Five[®] SFM at the concentration recommended by the manufacturer (18 mM). Each bottle of media (including EX-CELL[™] 405) was aseptically transferred to sterile 1L Nalgene PETG bottles and labeled with a code letter (Medium A, B, C or D). A 20 mL sample from each bottle was removed and cultured to verify sterility. All media bottles were packaged in the same shipping container and shipped overnight to ATG Laboratories, Inc., Eden Prairie, MN.

Adaptation to Serum-Free Media

Suspension cultures of High Five[™] cells, previously initiated in Grace's Insect Medium (supplemented with 10% FBS and 0.1% Pluronic[®] F-68) were adapted to serum-free conditions using a sequential adaptation (weaning) method. Cultures (100 mL culture volume per 250 mL spinner flask) were initiated at a cell density of 3×10^5 cells/mL in a 50:50 ratio of Grace's Insect Medium to each serum-free medium. All cultures were incubated at 27 C at 85 - 90 rpm, on the same magnetic stir plate. When cell densities reached at least 1×10^6 cells/mL, the cells were subcultured into a 25:75 ratio of Grace's Insect Medium to serum-free medium. When cell densities again reached 1×10^6 cells/mL, the cells were subcultured into a 20:80 ratio of Grace's Insect Medium to serum-free medium. Three more passes were performed in 100% serum-free medium at which point the cells were considered fully adapted. High Five[™] cells easily adapted to each of the serum-free preparations, with no lag phases or decreases in viability observed. Master cell stocks were generated by freezing at 1.0×10^7 cells/mL in a cryopreservation medium consisting of 7.5% dimethyl sulfoxide and 10% bovine serum albumin in a 50:50 mixture of fresh and conditioned medium.

Growth Studies

Three independent growth studies were initiated from the frozen stocks of adapted cells. Three vials of each master cell stock were thawed directly into spinner culture. Cell viability was determined immediately upon thawing and again at 24 hours post-thaw to ensure healthy cultures. Each culture was subcultured at least once for adaptation into suspension culture prior to commencement of the growth studies. Growth studies were initiated at a cell density of 3×10^5 cells/mL (100 mL culture volume per 250 mL spinner flask) and all cultures were incubated at 27 C at 85 - 90 rpm, on the same magnetic stir plate. Cells were subcultured every 3 days for a total of 4 passages. Cell densities and viabilities were determined daily by standard technique using trypan blue exclusion. After the last passage, cells were subcultured and subsequently infected with a recombinant baculovirus expressing β -galactosidase. Cells were infected at a multiplicity of infection (MOI) of 4.0 and density at time of infection was approximately 1×10^6 cells/mL. Four aliquots (1 mL each) of cells were harvested from each flask at 24, 48, 72 and 96 hours post-infection. Each aliquot was sedimented by centrifugation at 5000 rpm for 5 minutes at 4 C. The media supernatant was discarded and the cells washed with 1 mL of phosphate buffered saline. The cell pellets were stored at -80 C until the β -galactosidase assays were performed.

Protein Expression

Duplicate washed cell pellets from each of the 4 cultures were assayed using the β -galactosidase kit according to the manufacturer's directions. Each cell pellet was resuspended in Lysis Buffer (250 mM Tris (HCl) pH 8.0) and subjected to 3 freeze/thaw cycles (-80 C/37 C) to lyse the cells. The insoluble material in the lysate was removed by centrifugation at 13,000 rpm for 5 minutes at 4 C. The supernatants containing the aqueous-soluble proteins were transferred to fresh tubes. An aliquot of each supernatant was diluted 1:1000 (v/v) in Assay Buffer (10 mM NaH₂PO₄ pH 7, 10 mM KCl, 1 mM MgSO₄, 38 mM β -Mercaptoethanol). Two volumes

of each diluted supernatant (n = 16 total samples for each medium at each time point) were incubated at 37 C with the chromogenic substrate ONPG (ortho-nitrophenyl- β -D-galactopyranoside) (0.5 mg/mL). After 30 minutes the reaction was stopped by the addition of Stop Buffer (630 mM Na₂CO₃) and the absorbance at 405 nm of each sample was determined using an automated microplate reader (BIO-TEK Instruments, Inc., Model EL312e). β -galactosidase activity is reported as nanomoles (nmol) ONPG hydrolyzed/minute/mL of culture volume. Statistical analyses were performed by one-way analysis of variance (ANOVA) with either Tukey or Dunn's post-hoc tests, using SigmaStat[®] statistical software (Version 2.0, SPSS Science).

Results

Cell Density and Viability

For each growth study, cells in each of the 4 serum-free media were thawed from frozen master cell stocks and initiated as suspension cultures. Immediately upon recovery, and at 24 hours after thawing, the cell viability in each medium was determined. Table 1 shows the viability data for the

	HyQ [®] SFX-Insect [™]	EX-CELL [™] 405	Express Five [™] SFM	Ultimate Insect
Initial Recovery	94.5%	94.9%	96.3%	94.1%
24 Hours Post-Thaw	95.6%	96.4%	96.9%	94.7%

Three independent growth studies, comparing culture growth parameters in the 4 media types, were initiated from the frozen master cell stocks. The density of High Five[™] cells achieved in each medium during each growth study was very similar (see Figure 1), with most cultures reaching 3×10^6 cells/mL after 3 days (range 2.3-4.6 $\times 10^6$ cells/mL). Cell viability was consistently at or above 98% in each medium (range 93 - 100% viability).

Protein Expression

In each study, the production of soluble β -galactosidase was measured in each test culture at 24, 48, 72 and 96 hours post-infection (see Figure 2). The activity of β -galactosidase is plotted on a volume basis i.e. nmol ONPG hydrolyzed/minute/mL of culture. β -galactosidase was produced in each culture at every time point. Optimum β -galactosidase activity was measured at 48 hours post-infection, with cells cultured in EX-CELL[™] 405 producing the greatest amount of activity at both 48 and 72 hours.

Conclusions

The intent of this study was to compare the growth characteristics and recombinant protein expression of the BTI-TN-5B1-4 (High Five[™]) cell line using 4 commercially available serum-free medium. High Five[™] cells were easily adapted to serum-free suspension culture in each of the 4 media tested using a sequential weaning method. It was demonstrated that cells could easily be frozen and recovered with good viability. Cell density and viability were examined in 3 multiple-passage growth studies. Each serum-free medium supported rapid exponential growth with cultures reaching densities up to 4.6×10^6 cells/mL after 3 days. It was also shown that recombinant β -galactosidase was produced in every medium, at each time point examined. Maximal enzymatic activity occurred at 48 hours post-infection in all media. Cultures grown in EX-CELL[™] 405 produced the highest level ($p < 0.05$) of β -galactosidase enzymatic activity per milliliter of culture at both 48 and 72 hours post-infection.

Figure 1: Growth of BTI-TN-5B1-4 (High Five™) cells in spinner culture.

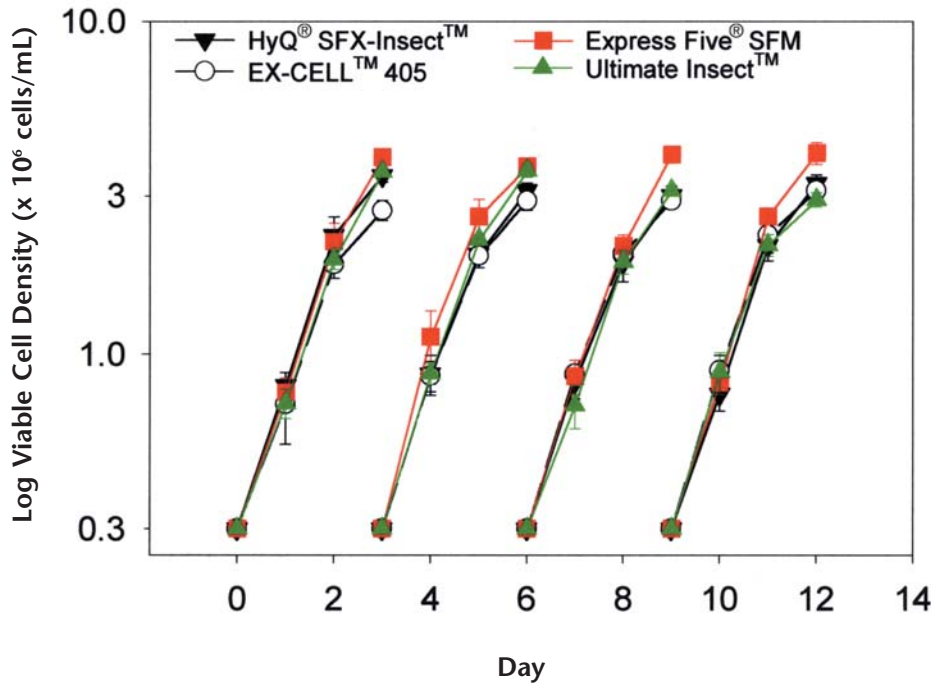


Figure 1: Growth of BTI-TN-5B1-4 (High Five™) cells in spinner culture. Cultures were seeded at 3×10^5 cells/mL, counted daily and subcultured every 3 days. Graph represents the mean \pm the SEM of 3 independent growth studies.

Figure 2: β -Galactosidase Activity

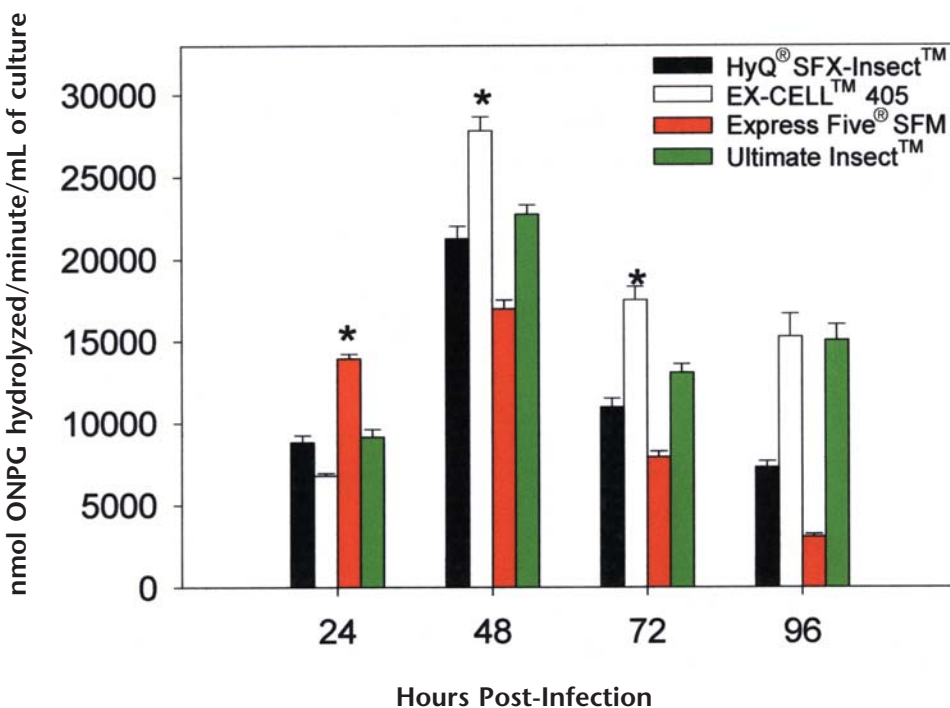


Figure 2: β -Galactosidase Activity. * Indicates the medium that produced significantly more β -galactosidase than all other media at that time point ($p < 0.05$). Data represent the mean \pm the SEM from 3 independent experiments.

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