GIBCO™
Invitrogen Corporation

EXPRESS FIVE® SFM

Optimized Serum-Free Medium for the Cultivation of the BTI-TN-5B1-4 Cell Line.

Cat. No.: 10486-025 1000 mL

without L-glutamine

Custom packaging available upon request.

Storage Condition: 2 to 8°C, in the dark.

FEATURES

- L-glutamine free to avoid problems associated with L-glutamine degradation, including ammonia accumulation, and to maximize shelf life.
- Superior growth of *BTI-TN-5B1-4 (HIGH FIVE™)*[1] cells when compared to other commercially available serum-free and serum supplemented media.
- Optimized for recombinant protein production via the *BTI-TN-5B1-4(HIGH FIVE™)* insect cell culture system (reported 1-10 fold increase over existing systems).
- Protein free.
- Capable of supporting long-term cell growth.
- Cells adapted to other commercially available serum-free media can be subcultured directly into EXPRESS FIVE SFM usually **with minimal** adaptation.

INTRODUCTION

EXPRESS FIVE SFM is a protein-free medium which supports increased cell growth of the BTI-TN-5B1-4 insect cell line as well as significantly increasing the production of recombinant proteins using the Baculovirus Expression Vector System (BEVS)[2]. For more information on the growth of insect cells and expression of recombinant proteins, see references 3-6.

BTI-TN-5B1-4 (HIGH FIVE™) cells grown in EXPRESS FIVE SFM achieve maximum cell densities of 5 to 6 x 10^6 cells/mL and rβ-Galactosidase expression up to 1.7 U/cell, a significant improvement over other media (see reference 1 and Figure 1).

BTI-TN-5B1-4 (HIGH FIVE™) cells have been carried long term in EXPRESS FIVE SFM and have compared favorably to cells grown in serum-supplemented and serum-free controls (Sf-900 II SFM). Infections with wild type and rAcNPV (expressing β-Galactosidase clone VL-941 have been completed. The results show a significant improvement in cell growth (Figure 1) and rDNA protein expression (Figure 2) over other serum-free medium or serum-supplemented media. Infections with either recombinant or wild-type AcNPV were done only when the cultures were in the exponential phase of growth and had reached densities of 1.5 to 2.5 x 10^6 cells/mL. Utility of EXPRESS FIVE SFM in larger scale culture systems was demonstrated with a 5L CELLIGEN™[7] bioreactor.

The medium contains a biologically active raw material, which is a critical growth promoting component. The color of the media may vary between manufactured lots due to the variability in carbohydrate processing of the raw material. The difference in coloration has no impact on medium performance. When using a new lot of this medium, observed cell growth may be initially higher or lower than routinely observed with the current lot of this medium. This is typically observed in the first cell passage and should resolve itself within one or two cell passages, as the cells adapt to the new lot of medium.

Figure 1

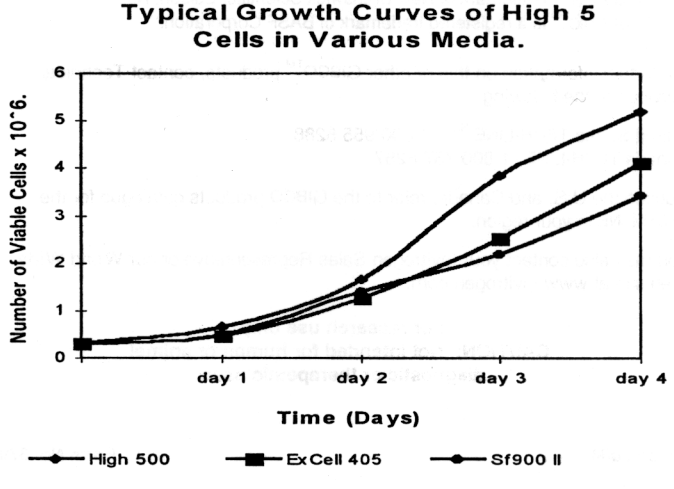
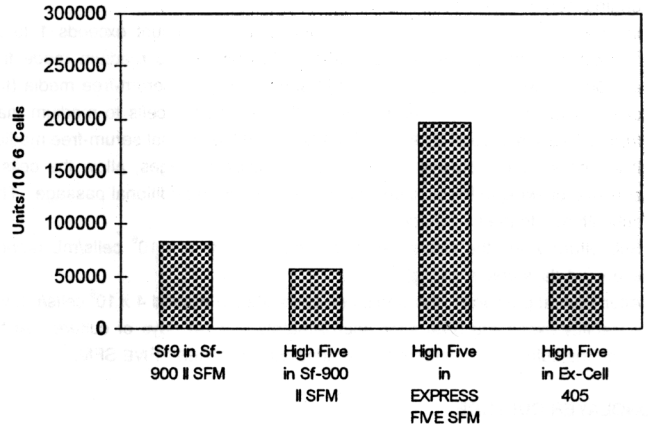


Figure 2

Beta-Galactosidase Expression in High Five vs. Sf9 Cells. Specific Productivity at 72 Hours Post-Infection.



CELL CULTURE PROCEDURES

General Information

BTI-TN-5B1-4 (High Five™) cultured cells have a doubling time of 16-30 hours in EXPRESS FIVE SFM. These cells may be easily grown in suspension culture, reaching maximum densities of up to 6 x 10^6 cells/mL in shaker culture. Cultures are incubated at 28 ± 0.5°C, in non-CO_2 equilibrated incubators.

EXPRESS FIVE SFM requires supplementation with L-glutamine prior to use. ASEPTICALLY ADD 90 mL of STERILE 200 mM L-Glutamine (Cat.No. 25030) per 1000 mL incomplete basal medium before use. There is ample room in the bottle for this supplementation. ***If L-glutamine is not required, add 90 mL of sterile distilled water, and adjust the osmolality to 365 mOsm using a solution of sterile NaCl.*** The addition of a surfactant such as PLURONIC® F-68[8] is not required.

I. SERUM-FREE CULTURE TECHNIQUES

Cell Adaptation Protocols-Introduction

There are two approaches to be considered when adapting cells to EXPRESS FIVE SFM: 1) Direct planting of cells from the original medium to EXPRESS FIVE SFM 2) Sequential adaptation or "weaning". It is critical that cell viability be at least 90% and the growth rate be in mid-logarithmic phase prior to initiating adaptation procedures. The use of these procedures would be similar for cells originally growing in serum-containing or serum-free medium.

A. Direct Adaptation

1. Cells growing in the original medium are transferred directly into prewarmed EXPRESS FIVE SFM following parameters set in III, steps 1-10.
2. When the cell density reaches 1 to 3 x 10^6 cells/mL (2-3 days postplanting), subculture to a density of 5 x 10^5 cells/mL.
3. When the cells are completely adapted to serum-free culture, they will reach a density in excess of 4 x 10^6 viable cells/mL after approximately 5 days culture.
4. Stock cultures of EXPRESS FIVE SFM adapted cells should be subcultured twice weekly when the viable cell count reaches 1 to 3 x 10^6 cells/mL with at least a 90% viability, subculture to a density of 3 x 10^5 cells/mL.

Note: If suboptimal performance is achieved using the direct adaptation method, use the sequential adaptation (weaning) method.

B. Sequential Adaptation / Weaning Procedure

1. Subculture *BTI-TN-5B1-4 (HIGH FIVE™)* cells growing in the original medium into a medium made from a 25:75 mixture of EXPRESS FIVE SFM and the original serum-free media.
2. Incubate according to III, steps 1-10 until viable cell count exceeds 1 to 2 x 10^6cells/mL and viability is above 85%. Subculture into medium made from a50:50 mixture of EXPRESS FIVE SFM and the original serum-free media (final cell density of 3 x 10^5). The next subculture seeds the cells to medium made from a 75:25 mixture of EXPRESS FIVE SFM and the original serum-free media.
3. If the cells do not survive at any of the adaptation stages, allow the cells to continue growing in the previous stage medium for an additional passage. Then subculture into the next stage.
4. Subculture when the viable cell count reaches 1 to 2 x 10^6 cells/mL (approx. every 3-4 days post planting).
5. After several passages, the viable cell count should exceed 4 x 10^6 cells/mL with a viability exceeding 85% after approximately four (4) days of culture. At this stage the culture is considered to be adapted to EXPRESS FIVE SFM.

II. MONOLAYER CULTURE

1. With a 10 mL pipette, aspirate medium and floating cells from a confluent monolayer and discard.
2. Add 4 mL of fresh complete medium to a 25 cm^2 flask (12 mL to a 75 cm^2 flask).
3. Resuspend cells by pipetting the medium across the monolayer with a Pasteur pipette (or equivalent device). These cells may attach tightly and may need to be dislodged with a wrist-snap motion.
4. Observe cell monolayer using an inverted microscope to ensure complete cell detachment from the surface of the flask.
5. Perform viable cell count on harvested cells (e.g., using trypan blue exclusion method).
6. Inoculate cells at 2 to 5 x 10^5 cells/cm^2.
7. Return cultures to incubator (28 ± 0.5°C).
8. On the third day post-planting, aspirate the spent medium from one side of the monolayer and re-feed the culture with fresh medium gently added to the side of the flask.

III. SUSPENSION CULTURE

1. Four (4) to six (6) confluent 75 cm^2 monolayer flasks are required to initiate a 100 mL culture (4-5 flasks for the suspension culture and one to be used as a backup).
2. Dislodge cells from the base of the flasks as described in II, steps 1-4. Pool the cell suspension and perform a viable cell count.
3. For culture volumes of 75-100 mL, use a 125 mL spinner vessel. For volumes of 150-200 mL, use a 250 mL vessel. For volumes of 25-50 mL, use a 125 mL shaker. For volumes of 50-100 mL, use a 250 mL shaker. Seed these vessels at approximately 3 x 10^5 viable cells/mL in complete medium.
4. Incubate the vessels at 28 ± 0.5°C, the spinner flasks at a constant stirring rate of 75 rpm, with the side arm caps loosened (about 1/4 turn) and the shaker flasks at 130-150 rpm, with the cap loosened 1/4 turn. Set the orbital shaker at 80-90 rpm for cultures maintained in medium supplemented with FBS.
5. Re-seed spinner cultures to approximately 3 x 10^5 cells per mL twice weekly in well-cleaned, sterile vessels.
6. If the cells clump during this phase, use the procedure described indetail in Section IV.B.3, Recovery of Cryopreserved Cells.

Note: *BTI-TN-5B1-4 (HIGH FIVE™)* cells are not anchorage dependent and may be transferred between monolayer and spinner/shaker culture repeatedly without noticeable perturbation of normal viability, morphology, or growth rate. Also, as cultures may be passage number dependent, fresh cultures should be established from frozen seed stocks every three (3) months.

IV. CRYOPRESERVATION

A. Freezing

1. Prepare desired quantity of cells in either spinner or shaker culture, harvesting in mid log phase of growth (day 3) with a viability of >90%.
2. Determine the viable cell count and calculate the required volume of cryopreservation medium (7.5% DMSO and 10% BSA in EXPRESS FIVE SFM) required to yield a final cell density of 0.5 to 1.0 x 10^7cells/mL.
3. Prepare the required volume of cryopreservation medium. Hold the medium at 4°C.
4. Pellet cells from culture medium at 75 x g for six (6) minutes. Re-suspend pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension to cryovials according to manufacturers specifications (i.e. 4.5 mL to a 5.0 mL cryovial).
6. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Frozen cells are stable indefinitely under liquid nitrogen.

B. Recovery

1. Recover cultures from frozen storage by rapid thawing a vial of cells in a 37°C water bath with shaking just until the medium thaws. Transfer the entire contents of the vial into the appropriately sized vessel so that the cells are seeded at 5 x 10^5 cells/mL of complete growth medium and incubate culture as per III, steps 1-4.
2. Maintain culture between 5 x 10^5 and 1 x 10^6 cells/mL for the first two subcultures after recovery; thereafter, returning to the normal maintenance schedule.
3. The incidence of clumping of the cells is decreased by modifying the subculture procedure slightly during the first few subcultures after recovery from a freeze (a time of increased clumping), or any time thereafter when necessary. This is accomplished by allowing the cells to settle for one to two minutes. The clumps quickly settle to the bottom. Aspirate the clumps into a 10 mL pipette and break up the clumps by pressing the pipette tip against the bottom of the flask and discharge the cells back into the medium. This can be repeated one or two more times. Count the cells and subculture or reincubate these cells. Smaller clumps can be removed by allowing the culture to settle for 30-60 seconds and taking the sample for counting from the upper layer of the culture. Count the cells, and take the aliquot necessary for subculture from the flask only after resuspending the cells and repeating the 30-60 second settling period. This would select for the cells that grow as a single cell in suspension.

References

1 HIGH FIVE™ Trademark of Invitrogen Corp., San Diego, CA.

2 Danner, D.J., Godwin, G., Kassay, K. and Gorfien, S. Abstract presented at the Baculovirus and Insect Cell Gene Expression Conference, Pinehurst, N.C. March 26-30 (1995).

3 Wickham, T.J., Davis, T., Granados, R.R., Shuler, M.L. and Wood, H.A. Screening of Insect Cell Lines for the Production of Recombinant Proteins and Infectious Virus in the Baculovirus Expression System. *Biotechnol.Prog.*, 8 **(5):** 391-396 (1992).

4 Wang, P., Granados, R.R. and Shuler, M.L. Studies on Serum-Free Culture of Insect Cells for Virus Propagation and Recombinant Protein Production. *J.Invert Path,* **59:**46-53 (1992).

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6 Davis, T., Wickham, T.J., McKenna, K.A., Granados, R.R. Shuler, M.L. and Wood, H.A. Comparative Recombinant Protein Production of Eight Insect Cell Lines. *In Vitro Cell. Dev. Biol.,* **29A:**388-390 (1993).

7 CELLIGEN™ Trademark of New Brunswick Scientific, Edison, NJ.

8 PLURONIC.® is a registered trademark of BASF Corporation.

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