GIBCO
invitrogen cell culture

Sf-900 II SFM

Optimized Serum-Free Medium for the Growth of Sf9 and other Invertebrate Cell Lines and Expression of Recombinant Proteins

Cat. No.: 10902 **500 mL**
1000 mL
10 L

Component -Deficient Media:
Cat. No. : 21012 **500 mL** **without L-methionine and L-cystine**

Custom packaging available upon request.
Storage Conditions: 2 to 8°C, in the dark.
Minimum shelf life after receipt: 2 months

NOTE: For catalog number 21012 please also refer to Component-Deficient Media Supplementation below.

FEATURES

- Superior growth of *Spodoptera frugiperda* (Sf9,Sf21), *Lymantria dispar* (Ld) and *Tricoplusia ni* (Tn-368) cells versus commercially available serum-free and serum supplemented media.
- Optimized for recombinant protein production via insect cell culture systems (2-10 fold increase over existing systems).
- Protein free
- Scalable in variety of bioreactors
- Capable of supporting long-term cell growth (>20 passages)
- Cells adapted to other commercially available serum-free media can be subcultured directly into Sf-900 II SFM usually **without** any further adaptation
- Supports high yields of wildtype AcNPV (occluded and non-occluded virus)

Introduction

Sf-900 II SFM is a protein-free medium which supports increased cell growth of a variety of insect cell lines as well as significantly increasing the production of recombinant proteins using the Baculovirus Expression Vector System[1,2]. For more information on the growth of insect cells and expression of recombinant proteins, see references 3-8.

Spodoptera frugiperda (Sf9) cells grown in Sf-900 II SFM achieve maximum cell densities of 9 to 12 x 10^6 cells/mL and rß-Galactosidase expression up to 500,000 U/mL, a significant improvement over competitor's formulations and the original Sf-900 formulation (see reference 1 and Figure 1). 20 to 100% increases in maximum cell densities are also observed with the *Lymantria dispar* (Gypsy moth) and *Trichoplusia ni* (Tn-368 Cabbage Looper) cell lines.

BEVS was initially developed by Smith et.al. of Texas A&M University[3]. Traditionally, Grace's TNM-FH insect cell culture medium supplemented with 10% FBS has been used for BEVS technology as described by Summers et.al. [4]. To date, over 200 recombinant (rDNA) proteins have been expressed in the Sf9 cell line infected with the *Autographa californica* nuclear polyhedrosis virus (AcNPV) [5]. Sf-900 II SFM is an improved serum-free medium designed for growth of Sf9 and other lepidopteran cell lines and production of insect virus and rDNA proteins. A number of institutions are currently utilizing the original Sf-900 formulation to produce their rDNA proteins in a serum-free environment. Although excellent Sf9 cell growth and rDNA protein expression is obtained for most BEVS applications, it has been determined that expression is rate limited at high cell densities (>4.0 x 10^6 cells/mL) owing to selective nutrient depletion[1]. Invitrogen development scientists have focused on further optimizing virus production and rDNA protein expression, which has resulted in the development of Sf-900 II SFM. The Sf-900 II SFM formulation, as compared to the original Sf-900 SFM, contains major changes in the amino acid, carbohydrate, vitamin and lipid components.

Sf9 as well as Tn-368 and Ld cells have been carried long term (>20 passages) in Sf-900 II SFM and compared to both serum-supplemented and serum-free controls (Sf-900 SFM). Infections with both wildtype and rAcNPV (expressing ß-Galactosidase clone VL-941 or EPO) have been completed. The results show a significant improvement in cell growth (Figure 2), virus production and rDNA protein expression (Figure 1) over other SFM or serum-supplemented media. Infections with either recombinant or wild-type AcNPV were done only when the cultures were in exponential growth and had reached densities of 1.5 to 2.5 x 10^6 cells/mL. Utility of Sf-900 II SFM in larger scale culture systems was demonstrated with a 5L Celligen™ bioreactor. Successful infections with rAcNPV were carried out producing rß-Gal (Figure 3) and rEPO (Figure 4).

This 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 Sf900 II SFM, observed cell growth may be initially higher or lower than routinely observed with the current lot of Sf900 II SFM. 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.

CELL CULTURE PROCEDURES

GENERAL INFORMATION

Spodoptera frugiperda (Sf9) cultured cells (ATCC #CRL 1711) have a doubling time of 20-24 hours in Sf-900 II SFM. A monolayer will reach confluency in 5-6 days with a medium change on day three. These cells may be easily grown in suspension culture, reaching maximum densities of up to 1 x 10^7 cells/mL in shaker culture. Cultures are incubated at 28°C ± 0.5°C.

Sf-900 II SFM is a complete, ready to use medium. Do not add L-Glutamine or surfactants such as PLURONIC® F-68.

I. SERUM-FREE CULTURE TECHNIQUES

Cell Adaptation Protocols - Introduction

There are two approaches to be considered when adapting cells to Sf-900 II SFM: 1) Direct planting of cells from medium containing serum to Sf-900 II 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 adaption procedures.

Materials and Equipment:

Incubator capable of maintaining 28°C ± 0.5°C, and large enough to accommodate orbital shaker with dual platform.
Insect cell culture growth medium: Sf-900 II SFM and currently used complete medium.
Microscope, Hemocytometer
Electronic Cell Counter
Low speed centrifuge
Pipettes: 1 mL, 5 mL, 10 mL, 25 mL
Pasteur pipettes
250 mL sterile disposable Erlenmeyer flasks

A. Direct Adaptation

1) Cells growing in 5% to 10% FBS supplemented medium are transferred directly into prewarmed Sf-900 II SFM following parameters set in III, steps 1-10.
2) When the cell density reaches 1-3 x 10^6 cells/mL (4-6 days post planting), subculture to a density of 3 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 7 days culture.
4) Stock cultures of 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.

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

B. Sequential Adaptation / Weaning Procedure

1) Subculture Sf9 cells grown in conventional medium with 5-10% serum into a 50:50 ratio of Sf-900 II SFM and the original serum supplemented media.
2) Incubate according to III, steps 1-10 until viable cell count exceeds 6 x 10^5 cells/mL. Subculture by mixing equal volumes of culture and fresh Sf-900 II SFM.
3) Continue to subdivide the culture in this manner until the calculated serum complement falls below 0.1%, cell viability is approximately 85% and a viable count in excess of 6 x 10^5 cells/mL is achieved.
4) Subculture when the viable cell count reaches 1-2 x 10^6 cells/mL (approx. every 4-6 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 7 days of culture. At this stage the culture is considered to be adapted to Sf-900 II SFM.

II. MONOLAYER CULTURE

Materials and Equipment:

Those required from Part I above
T-Flasks (25 and 75 cm^2)

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).
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 1.2 x 10^6 cells/25 cm^2 flask or 3.6 x 10^6 cells/75 cm^2 flask.
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. SPINNER CULTURE

Materials and Equipment:

Those required from Part I
100 or 250 mL Spinner flasks
Stirring platform

1) Recalibrate the gradation marks on commercial spinner flasks using a graduated cylinder or volumetric flasks as a reference. Calibration is performed with the impeller apparatus removed from the vessel.
2) Ensure that impeller mechanisms rotate freely and do not contact vessel walls or base (Sf9 cells are sensitive to physical shearing). Adjust the spinner mechanism so that paddles clear sides and bottom of the vessel (adjust prior to autoclaving).
3) Four (4) to six (6) confluent 75 cm^2 monolayer flasks are required to initiate a 100 mL culture (4-5 flasks for the spinner culture and one to be used as a backup).
4) Dislodge cells from the base of the flasks as described in II, steps 1-4.
5) Pool the cell suspension and perform a viable cell count.
6) Dilute the cell suspension to approximately 3 x 10^5 viable cells/mL in complete medium.
7) For culture volumes of 75-100 mL, use a 100 mL spinner vessel. For volumes of 150-200 mL, use a 250 mL vessel.
8) Stock cultures should be maintained in a 150 mL culture in a 250 mL spinner vessel. The top of the paddles will be slightly above the medium, which provides additional aeration to the cultures.
9) Atmospheric gas equilibration is accomplished by loosening the side arm caps on the vessels (about 1/4 turn).
10) Incubate spinner vessels at 28 ± 0.5°C at a constant stirring rate of 75 rpm.
11) Re-seed spinner cultures to approximately 3 X 10^5 cells per mL twice weekly in well-cleaned, sterile vessels.
12) Once every two weeks spinner cultures may be gently centrifuged at 100 x g for 5 minutes and resuspended in fresh medium to reduce accumulation of cell debris and toxic metabolic by-products.

IV. SHAKER CULTURE

Materials and Equipment:

Those required from Part I
Orbital shaker with dual platform for 250 mL Erlenmeyer flasks
Disposable 250 mL Erlenmeyer flasks

Protocol

A. Shaker Apparatus

1. Orbital shaker apparatus must have a capacity of up to 135 rpm.
2. The standard flask employed is the 250 mL disposable sterile Erlenmeyer (especially when working with serum-reduced or serum-free medium).
3. The orbital shaker/flask assembly should be maintained in a 28 ± 0.5°C non-humidified, non-gas regulated environment.
4. Aeration is accomplished by loosening the cap approximately 1/4 turn (within the intermediate closure position). In this condition, there is no oxygen limitation to the cells and they therefore proliferate to maximal rates.

B. Culture Manipulation

1. Inoculate a 250 mL Erlenmeyer flask with 100 mL of complete medium containing 3 x 10^5 viable cells per mL.
2. Set the orbital shaker at 80-90 rpm for cultures maintained in medium supplemented with FBS. For cultures in serum-free medium, the shaker should be maintained at 125-135 rpm.
3. Subculture to approximately 3 x 10^5 cells/mL twice weekly.
4. Every three weeks, cultures may be gently centrifuged at 100 x g for 5 minutes and pellets resuspended in fresh medium to reduce accumulation of cell debris and toxic metabolic by-products.

Note: Sf9 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. However, as cultures may be passage number dependent, fresh cultures should be established from frozen seed stocks every three months.

V. CRYOPRESERVATION

Materials and Equipment:

DMSO
Cell cryopreservation vials
Fresh Sf-900 II SFM
Cell culture grade Bovine Serum Albumin

A. Freezing

1. Prepare desired quantity of cells in either spinner or shaker culture, harvesting in mid log phase of growth at a viability of >90%.
2. Determine the viable cell count and calculate the required volume of cryopreservation medium required to yield a final cell density of 0.5 to 1.0 x 10^7 cells/mL.
3. Prepare the required volume of cyropreservation medium consisting of 7.5% DMSO and 10% BSA in fresh Sf-900 II medium. Hold medium at +4°C.
4. Pellet cells from culture medium at 100 x g for 6 minutes. Re-suspend pellet in the determined volume of +4°C cryopreservation medium.
5. Incubate cell suspension at +4°C for 30 minutes (until well chilled).
6. Dispense aliquots of this suspension to cryovials according to manufacturers specifications (i.e 4.5 mL to a 5.0 mL Cryovial).
7. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures.
8. 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. Transfer the entire contents of the vial into a 250 mL shaker flask containing 100 mL complete growth medium and incubate culture as per IVA, steps 1-4.
2. Maintain culture between 3 x 10^5 and 1 x 10^6 cells/mL for the first two subcultures after recovery; thereafter returning to the normal maintenance schedule.

Component-Deficient Media Supplementation

When using catalog number 21012, the following supplementation concentration will be consistent with the original formulation:

21012 L-methionine at 1.000 g/L
L-cystine•2Na at 0.150 g/L

References:

1 Weiss, S.A., Godwin, G.P., Gorfien, S.F., Whitford, W.G.. Serum-Free Media. In: *Insect Cell Culture Engineering*. Eds., Daugulis, A., Faulkner, P. and Goosen, M.F.A. Pub., Marcel Dekker, Inc. (In Press).

2 Weiss, S.A., DiSorbo, D.M., Whitford, W.G. and Godwin, G.P. Improved Production of Recombinant Proteins in High Density Insect Cell Culture. *In Vitro Cell Dev. Biol.* (abs) **27**(3):42a (1991).

3 Smith, G.E., Summers, M.D. and Fraser, M..J. Production of Human B-interferon in Insect Cells Infected with a Baculovirus Expression Vector. *J Molecular and Cellular Biology*, **3**(12):2156- 2165 (1983).

4 Summers,M.D. and Smith,G.E. A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures. *Texas Agricultural Experiment Station Bulletin* No. 1555, (May 1987).

5 Luckow, V.A. Cloning and Expression of Heterologous Genes in Insect Cells with Baculovirus Vectors. In: *Recombinant DNA Technology and Applications*. Eds: Prokop, Bajpaj, Ho. Chapter 4, p97-153. ISBN:0-07-029075. McGraw Hill. New York (1991).

6 Weiss, S.A., Gorfien, S., Fike, R., DiSorbo, D. and Jayme, D. Large-Scale Production of Proteins Using Serum-Free Insect Cell Culture. Ninth Australian Biotechnology Conference, in *Biotechnology: TheScience and the Business*. pp. 220-231, Gold Coast, Queensland, Australia, (Sept. 24-27, 1990).

7 Weiss, S.A., Belisle, B.W., DeGiovanni, A., Godwin, G., Kohler, J. and Summers, M.D. Insect Cells as Substrates for Biologicals. In: *International Association for Biological Standardization, Symposium on Continuous Cell Lines as Substrates for Biologicals*. Arlington, Virginia USA, 1988, Dev. Biol. Standard, Vol. 70, pp. 271-289 (S. Karger, Basel 1989).

8 Weiss, S.A., DeGiovanni, D.M. and Godwin,G.P. Use of Insect Cells in Biotechnology. *In Vitro Cell. Dev. Biol.* (abs) **24**(3):53a (1988).

9 Vaughn, J.L. and Weiss, S.A.. Large Scale Propagation of Insect Cells. In: *Large Scale Mammalian Cell Culture Technology*. Ed, A.S. Lubiniecki. p597-617. Marcel Dekker, Inc. New York. (1991).

For further information on this or other GIBCO® products, contact Technical Services at the following:

United States TECH-LINE SM: 1 800 955 6288
Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

For research use only.
CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

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Figure 1. β-GALACTOSIDASE EXPRESSION IN Sf9 CELLS GROWN IN SF-900 II SFM VS. SERUM-FREE AND SERUM SUPPLEMENTED CONTROLS

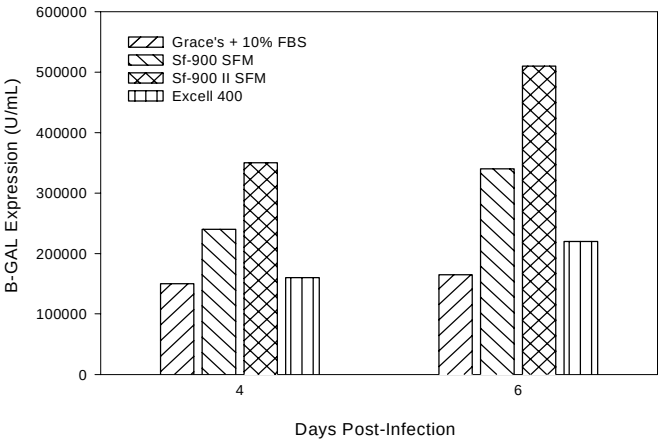

Figure 1. 250 mL shake flask cultures were seeded at 3 x 10E5 cells/mL (100 mL volume). The cultures were infected on day 4 post-planting with rAcNPV at a MOI of 0.50 when the cell densities reached 3 x 10E6 cells per mL. β-Galactosidase was assayed on days 4 and 6 post-infection.

Figure 2. Sf9 CELL GROWTH IN SERUM FREE AND SERUM SUPPLEMENTED MEDIA

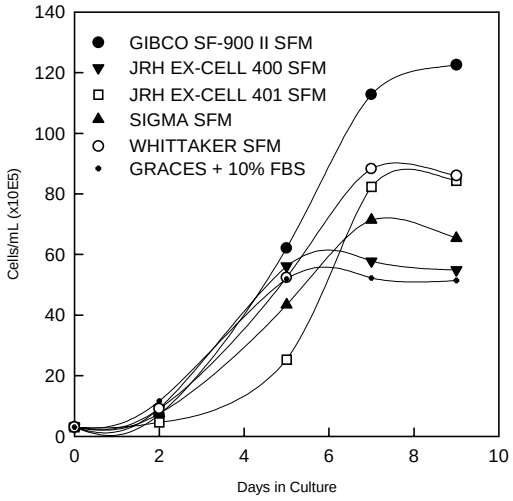

Figure 2. Sf9 cells growing in Graces Supplemented Media + 10% FBS were adapted to growth in the above insect serum-free media. After 5 passages serum-free. 250 mL erlenmeyer shake flasks were seeded at 3 x 10E5 viable cells/mL in 100 mLs growth media. Cultures were incubated at 27°C with a shaking speed of 135 rpm. Cell growth was monitored for nine days post-planting.

Figure 3. ß-GALACTOSIDASE PRODUCTION IN A 5L CELLIGEN BIOREACTOR USING Sf-900 II SFM

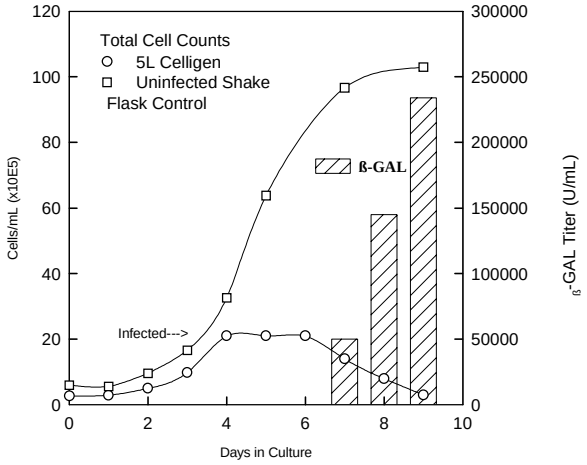

Figure 3. CELLIGEN™ was seeded with Sf9 cells at 2 x 10E5 cells/mL and infected on day 4 post-planting with rAcNPV (construct VL-941) at MOI of 0.50 when the culture reached 2 x 10E6 cells/mL. Bioreactor was sampled daily post-infection for β-GAL expression.

Figure 4. rEPO PRODUCTION IN SF-900 II SFM 5L CELLIGEN™

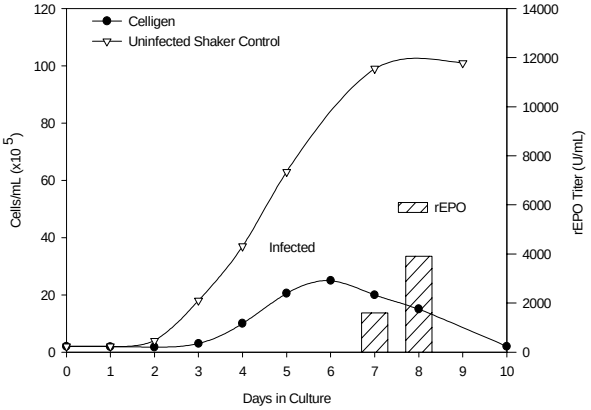

Figure 4. CELLIGEN™ was seeded with Sf9 cells at 2 x 10E5 cells/mL and infected on day 5 post-planting with rAcNPV at a MOI of 0.50 when the cell density reached 2.5 x 10E6 cells/mL. The culture was sampled daily post infection for rEPO.