GIBCO™
Invitrogen Corporation

Drosophila - SFM

Optimized Serum-Free Medium for the Growth of Drosophila melanogaster cells.

Cat. No.: 10797 **500 mL**
1000 mL

without L-Glutamine.
Add 45 mL of 200 mM L-Glutamine per 500 mL prior to use.

Custom packaging available upon request.
Storage Conditions: 2 to 8°C, in the dark.

Intended Use
Drosophila-SFM is a protein-free medium which supports increased cell growth of the *Drosophila melanogaster, D.Mel-2* (ATCC #CRL 1963) insect cell line. For more information on the growth of insect cells and expression of recombinant proteins, refer to references 1, 2. It is intended for laboratory research use only.

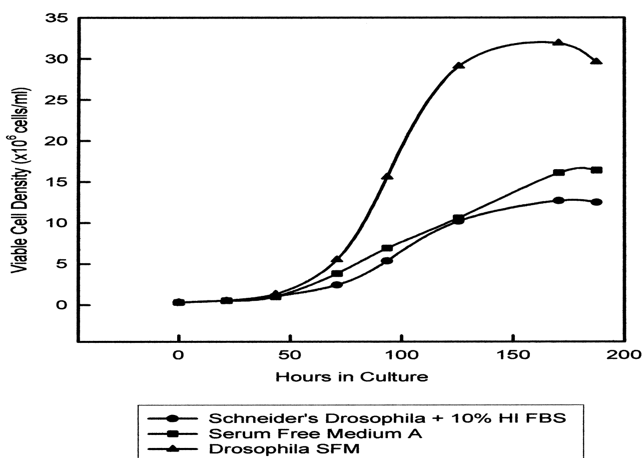
FEATURES

- L-Glutamine free to avoid problems associated with L-Glutamine degradation and to maximize shelf life.
- Superior growth of the D.Mel-2, *Drosophila melanogaster* cell line when compared to other commercially available serum-free and serum supplemented media.
- Protein free.
- Capable of supporting long-term serum-free cell growth.
- Cells adapted to other commercially available serum-free media can be subcultured directly into Drosophila-SFM **with minimal** adaptation.

Introduction
Drosophila melanogaster cells grown in Drosophila-SFM achieve doubling times of 12 to 16 hours and maximum cell densities up to 33 x 10^6 cells/mL, a significant improvement over other media (refer to Figure 1).

Figure 1. *Drosophila* Cell Growth in Serum-Free vs. Serum Supplemented Media.

***D.Mel-2* cells adapted to suspension culture in 50 ml shake flask cultures.**

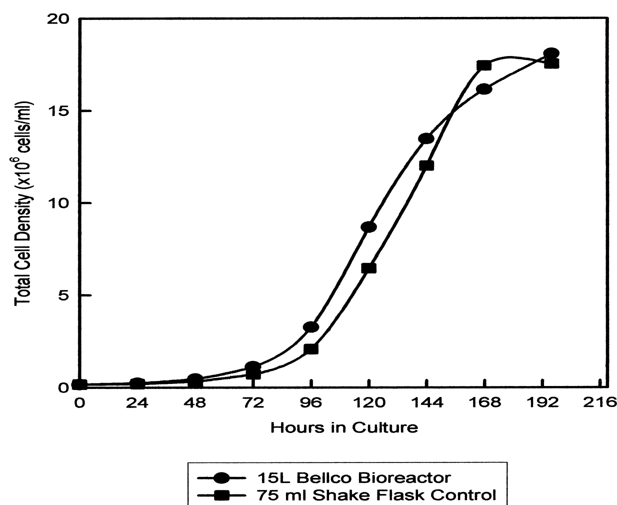


Drosophila melanogaster cells have been carried long term (>25 consecutive passages) in Drosophila-SFM. Cell growth rates and maximum densities were consistently superior to that of cells grown in serum-supplemented Schneider's Drosophila Medium and serum-free controls (Sf-900 II SFM). Utility of Drosophila-SFM in larger scale culture systems has been demonstrated in a 15L Bellco bioreactor (refer to Figure 2).

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 2. *Drosophila* Cell Growth in a 15L Bellco Bioreactor System

***D.Mel-2* cells adapted to serum-free suspension culture in DROSOPHILA SFM.**



CELL CULTURE PROCEDURES

General Information
Drosophila melanogaster cells have a mid-log phase doubling time of 12-16 hours in Drosophila-SFM. These cells are easily grown in suspension culture, reaching maximum densities of 20 to 33 x 10^6 cells/mL in shake flask culture. Cultures are incubated at 27°C ± 0.5°C, in non-CO_2 equilibrated incubators.

Drosophila-SFM requires supplementation with L-Glutamine prior to use. ADD 45 mL of 200 mM L-Glutamine (Cat. No. 25030) per 500 mL incomplete basal medium prior to use. There is ample room in the bottle for this supplementation. ***If L-Glutamine is not required, add 45 mL of sterile distilled water per 500 mL incomplete basal medium and adjust the osmolality to 365 using a solution of sterile NaCl.*** **The addition of a surfactant such as PLURONIC® F-68 is not required.**

I. SUSPENSION AND SERUM-FREE CULTURE ADAPTATION TECHNIQUES

Cell Adaptation Protocols - Introduction
Drosophila melanogaster cells are not anchorage dependent and may be transferred between monolayer and spinner/shaker suspension culture repeatedly without noticeable perturbation of normal viability or morphology and only a minimal short term (1 to 2 passage) reduction in growth rates. However, we recommend that one adapt and maintain their serum-supplemented or serum-free *Drosophila* cultures to suspension, for easier maintenance and subculturing. In addition, the serum-free adaptation protocols below were developed for use with suspension adapted cultures.

There are two approaches to be considered when adapting cells to Drosophila- SFM: 1) Direct planting of cells from the original medium to Drosophila-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 is recommended for cells originally growing in either serum-containing or serum-free medium formulations.

A. Adaptation to Suspension Culture

1) Two to three confluent T-75 cm^2 monolayer flasks are required to initiate a 50 mL spinner or shaker culture and to seed/maintain a backup monolayer culture.
2) Dislodge cells from the surface of the flasks as described below in Section II, steps 1-4. Pool the cell suspension and perform total and viable cell counts.
3) Monolayer cultures growing in serum-free or serum-supplemented media can then be seeded directly into complete, prewarmed serum-free or serum-supplemented media and maintained in shaker or spinner flasks following the parameters described in Sections III or IV below.
4) Suspension cultures will attain normal growth rates and densities in 1 to 2 passages.

Note: For cells in serum-supplemented media, we recommend supplementation with 0.05 to 0.10% PLURONIC® F-68 (available as a 10% liquid, Cat. No. 24040).

B. Direct Adaptation to Drosophila-SFM

1) Subculture *Drosophila melanogaster* cells, adapted to suspension culture and growing in the original serum-supplemented or serum-free media, directly into Drosophila-SFM at 5×10^5 viable cells/mL. Incubate and maintain suspension culture following the parameters described in Section III or IV below.
2) When the cell density reaches 5 to 10×10^6 viable cells/mL, subculture to a density of 5×10^5 viable cells/mL. Maintain as described above.
3) After 3 to 5 passages in Drosophila-SFM, *Drosophila* cells will attain population doubling times of 12 to 16 hours, saturation or maximum densities of 15 to 10×10^6 cells/mL after approximately 5 days in culture, and viabilities of >90%. At this point the culture is considered to be adapted to Drosophila-SFM.
4) Stock suspension cultures of Drosophila-SFM adapted cells should be subcultured when the viable cell density reaches 5 to15 $\times 10^6$ cells/mL, with a seeding density of 3×10^5 cells/mL.
Refer to Sections III and IV below.

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

C. Sequential Adaptation / Weaning to Drosophila-SFM

1) Subculture *Drosophila melanogaster* cells, adapted to suspension culture and growing in the original serum-supplemented or serum-free media, into medium composed of a 25:75 mixture of Drosophila-SFM and the original media, at 5×10^5 viable cells/mL. Incubate and maintain suspension culture following the parameters described in Sections III or IV below.
2) When the cell density exceeds 5×10^6 cells/mL, subculture into medium composed of a 50:50 mixture of Drosophila-SFM and the original media, at 5×10^5 viable cells/mL. Maintain as described above.
3) Seed the cells into medium composed of a 75:25 mixture of Drosophila-SFM and the original media, at 5×10^5 viable cells/mL. Maintain as described above.
4) Seed the cells into medium composed of a 90:10 mixture of Drosophila-SFM and the original media, at 5×10^5 viable cells/mL. Maintain as described above.
5) Seed the cells into Drosophila-SFM, at 5×10^5 viable cells/mL. Maintain as described above.
6) If the cells do not survive any of the adaptation stages or the cell viability drops below 75%, maintain the cells in the previous medium mixture for an additional passage.
7) After 3 to 5 passages in Drosophila-SFM, *Drosophila* cells will attain population doubling times of 12 to 16 hours, saturation or maximum densities of 15 to 10×10^6 cells/mL after approximately 5 days in culture, and viabilities of >90%. At this point the culture is considered to be adapted to Drosophila-SFM.
8) Stock suspension cultures of Drosophila-SFM adapted cells should be subcultured when the viable cell density reaches 5 to 15×10^6 cells/mL, with a seeding density of 3×10^5 cells/mL.
Refer to Sections III and IV below.

II. MONOLAYER CULTURE

1) With a 10 mL pipette, aspirate medium and floating cells from a confluent monolayer and discard.
2) Add 4 to 6 mL of fresh complete medium to a 25 cm^2 flask (12 to 15 mL to a 75 cm^2 flask).
3) Resuspend cells by pipetting the medium across the monolayer with a sterile 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 2 to 5×10^4 cells/cm^2.
7) Return cultures to incubator (27° ± 0.5°C).
8) Subculture when the monolayer is 80 to 100% confluent, as described in steps 1 to 7 above.

III. SPINNER CULTURE

1) For culture volumes of 75-100 mL, use a 100 mL spinner vessel. For volumes of 150-200 mL, use a 250 mL spinner vessel.
2) Inoculate vessel with appropriate amount of complete medium and seed at approximately 3×10^5 viable cells/mL.
3) Incubate spinner vessels at 27 ± 0.5°C in a non-humidified, non-CO_2 regulated incubator at a constant stirring rate of 75-90 rpm, with the side arm caps loosened (approximately 1/4 turn). The stirring rate may need to be adjusted to accommodate various impeller styles.
4) Subculture to approximately 3×10^5 cells per mL, when the density reaches 5 to 10×10^6 viable cells/mL.

IV. SHAKER CULTURE

1. For culture volumes of 25-50 mL, use a 125 mL shake flask. For volumes of 50-100 mL, use a 250 mL shake flask.
2. Inoculate a 250 mL Erlenmeyer flask with 75 to 100 mL of complete medium and seed at approximately 3×10^5 viable cells/mL.
3. Set the orbital shaker speed at 130-150 rpm. Incubate shake flasks at 27 ± 0.5°C in a non-humidified, non-CO_2 regulated incubator, with the cap loosened (approximately 1/4 turn).
4. Subculture to approximately 3×10^5 viable cells/mL, when the density reaches 10 to 15×10^6 viable cells/mL.

V. CRYOPRESERVATION

A. Freezing

1. Prepare desired quantity of cells in either spinner or shaker culture, harvesting in mid-log phase of growth (day 3 or 4) with a viability of >90%.
2. Determine the viable cell count and calculate the required volume of cryopreservation medium (7.5% DMSO in Drosophila-SFM) required to yield a final cell density of 0.5 to 1.0×10^7 viable cells/mL. A 50:50 mix of conditioned: fresh medium (with 7.5% DMSO) may also be used.
3. Prepare the required volume of cyropreservation medium. Hold the medium at +4°C.
4. Pellet cells from culture medium at 1000 rpm for 5 minutes. Re-suspend pellet in the pre-determined volume of +4°C cryopreservation medium.
5. Dispense aliquots of this suspension into 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 storage.

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

B. Recovery

1) Recover cultures from frozen storage by rapidly thawing a vial of cells in a 37°C water bath with shaking until the medium completely thaws. Aseptically transfer the entire contents of the vial into the appropriately sized vessel so that the cells are seeded at >5×10^5 viable cells/mL in complete Drosophila-SFM. Incubate culture as per Sections III or IV above.
2) Maintain culture between 5×10^5 and 10×10^6 viable cells/mL for the first two subcultures after recovery; thereafter returning to the normal maintenance schedule.

PLURONIC® is a registered trademark of BASF Corporation.

References:

1. Culp, J.S., Johansen, H., Hellmig, B., Beck, J., Matthews, T.J., Delers, A. and Rosenberg, M. Regulated expression allows high level production and secretion of HIV-1 gp120 envelope glycoprotein in *Drosophila* Schneider cells. *Biotechnology,* Vol. 9, 173-177 (1991).
2. Incardona, J.P. and Rosenberry, T.L. Construction and characterization of secreted and chimeric transmembrane forms of *Drosophila* acetylcholinesterase: A large truncation of the C-terminal signal peptide does not eliminate glycoinositol phospholipid anchoring. *Molecular Biology of the Cell*, Vol. 7, 595-611 (1996).

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

United States TECH-LINE ℠ : 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.

June 2001 **Form No. 3870**