GIBCO™
Invitrogen Corporation

Sf9 Cells

Cat. No. 11496-015

Shipping and Storage

Cells are supplied in a cryogenic vial containing 1.5 x 10^7 viable cells in a volume of 1.5 mL. **Store in liquid nitrogen (vapor phase).**

Caution

This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

General Media Requirements

Suspension or adherent culture: Use Sf-900 II SFM as is. Sf-900 II SFM contains L-Glutamine and does not require additional supplements. Protect media from light.

Note: Antibiotics are not recommended; however, 5 mL/L of Penicillin-Streptomycin may be used when required.

Thawing Cells

Store frozen Sf9 cells in liquid nitrogen (vapor phase) until ready to use. Frozen cells are supplied in and may be thawed directly into Sf-900 II SFM. Use the following procedure to thaw cells.

Note: We recommend thawing Sf9 cells into suspension culture in shake flasks. Do not thaw Sf9 cells directly into spinner vessels. For spinner culture applications, thaw cells into shake flasks using the procedure below. Carry and expand cells in shake flasks for 2 to 3 passages, then seed cells into spinner vessels.

1. Rapidly thaw frozen vial in a 37°C water bath. Triturate and transfer the entire contents of the cryovial into a 125 mL shake flask containing 27 mL of pre-warmed Sf-900 II SFM, and incubate in a 28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room on an orbital shaker platform rotating at 125-150 rpm. Loosen caps of flasks to allow oxygenation/aeration.
2. Once the culture density has reached >2 x 10^6 viable cells/mL, determine viable and total cell counts (see procedure).
3. Expand Sf9 cultures by seeding shake flasks at 3 to 5 x 10^5 viable cells/mL by diluting cells in pre-warmed growth medium. See **Subculturing Cells** to maintain and subculture Sf9 cells in suspension or adherent culture.

Note: We recommend subculturing cells for a minimum of 3 passages before use in other applications.

Determining Cell Density and Viability

Follow the procedure below to determine viable and total cell counts.

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.
2. Determine viability using the trypan blue exclusion method.
3. Determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

Subculturing Cells

Use the recommended conditions, procedure, and tips to subculture Sf9 cells. We recommend thawing a fresh, low-passage culture of frozen Sf9 cells every 3 months or 30 passages. For more information about maintaining suspension and adherent cultures, refer to the *Guide to Baculovirus Expression Vector Systems (BEVS) and Insect Cell Culture Techniques*, which is available for downloading from our Web site (www.invitrogen.com).

Recommended Conditions

	Suspension Cultures	Adherent Cultures
Cell density	>2 x 10^6 viable cells/mL	>80% confluent
Culture vessel	125 or 250 mL disposable, sterile Erlenmeyer flask containing 35-50 mL or 75-100 mL total working volume of cell suspension, respectively **Note:** Glass flasks without baffles may be used, but be sure to clean flasks thoroughly after each use to avoid potential toxicity.	T-75 cm^2 to T-162 cm^2 disposable sterile T-flasks. Dilute cells in a total working volume of 15-20 mL for T-75 cm^2 flasks and 40-50 mL for T-162 cm^2 flasks
Seeding density	3 to 5 x 10^5 viable cells/mL	2 to 5 x 10^4 viable cells/cm^2
Incubation conditions	28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room on an orbital shaker platform rotating at 125-150 rpm; loosen caps to allow for oxygenation/aeration	28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room; loosen caps to allow for oxygenation/ aeration

Subculturing Procedure

1. **Suspension cultures:** Proceed to Step 2. **Adherent cultures:** Remove medium and floating cells from a confluent monolayer and discard. Add 10 mL of fresh growth medium to a T-75 cm^2 flask (20 mL to a T-162 cm^2 flask). Displace cells from the flask's surface by rapping the flask sharply against your hand 3 or 4 times (>75% of the cells should be detached from the surface of the flask). Transfer the cell suspension into a centrifuge tube.
2. Determine viable and total cells counts (see procedure).
3. Seed cells at the recommended density (see table), diluting in pre-warmed growth medium. Put flasks in incubator with caps loosened to allow for oxygenation/aeration.

Subculturing Tips

Suspension cultures: To reduce accumulation of cell debris and metabolic waste by-products in shaker cultures, we recommend gently centrifuging the cell suspension at 100 x g for 5 to 10 minutes and resuspending the cell pellet in fresh Sf-900 II SFM once every 3 weeks.

Adherent cultures: For slower growing adherent cultures, we suggest removing spent media and replacing with fresh growth media every 3 to 4 days until Sf9 cells are ready to subculture.

Scaling-Up Sf9 Cells into Spinner Culture

You may scale-up the Sf9 cultures in spinner flasks using the guidelines below. Note that the appropriate spinner or impeller speed and seeding density should be determined and optimized for each system. For spinners >500 mL, use a vessel that provides for gas sparging.

- **Spinner culture volume:** The total culture volume should not exceed 60% of the indicated volume of spinner for proper aeration (*e.g.* a 250 mL spinner should not contain >150 mL of culture).
- **Spinner or impeller speed:** Determine the optimum impeller speed for your spinner vessel depending on your needs. To reduce loss of viability due to cell shearing, make sure that the impeller blade rotates freely and does not contact vessel walls or base.
- **Seeding density:** We use optimized seeding densities of 3 to 5 x 10^5 viable cells/mL and subculture cells when they reach a density of >2 x 10^6 viable cells/mL.

Freezing Cells

Recommended Conditions

- Freeze cells at a density of ≥1 x 10^7 viable cells/mL.
- Use a freezing medium composed of 50% fresh growth medium and 50% conditioned growth medium (day 2 to 4 cell conditioned media collected from Sf9 cultures during subculture procedure) and DMSO to a final concentration of 7.5%. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and chill at 4°C until use. Discard any remaining freezing medium after use.

Freezing Procedure

1. Grow the desired quantity of Sf9 cells in shake or spinner flasks, harvesting when the cells are in mid-log exponential growth and have a viability of >90%.
2. Determine viable and total cell counts (see procedure on the previous page) and calculate the volume of freezing medium required to yield a final cell density of ≥1x10^7 viable cells/mL.
3. Prepare the required volume of freezing medium (see above).
4. Centrifuge cells from cell suspension (Step 1) at 100 x g for 5 to 10 minutes. Aseptically decant supernatant and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.
5. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications (*i.e.* 1.5 mL in a 2 mL cryovial).
6. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
7. Transfer frozen vials to liquid nitrogen (vapor phase) storage.

Note: You may check the viability and recovery of frozen cells 24 hours after storing vials in liquid nitrogen by following the procedure outlined in **Thawing Cells**, previous page.

Transfection

For optimal results, we recommend using Cellfectin® Reagent available from Invitrogen for transfection. Refer to the manual accompanying the product for instructions. Note that if you use Cellfectin® Reagent, you may transfect cells directly in Sf-900 II SFM. Other transfection reagents are suitable.

General Information

The Sf9 insect cell line is a clonal isolate derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf-21-AE[1,2], and is a suitable host for expression of recombinant proteins from baculovirus expression systems[3,4] (*e.g.* Invitrogen's Bac-to-Bac® and Bac-N-Blue™ Expression Systems).

The Sf9 cell line exhibits the following general features:

- Prepared from low passage Master Cell Bank cultures that are only 40 to 50 total passages and 10 to 20 passages serum-free.
- Adapted to serum-free suspension growth in Sf-900 II SFM, a serum-free medium optimized for growth of Sf9 and other invertebrate cell lines[5]. **Note:** Cells also grow well in traditional media supplemented with serum[6] (*i.e.* Grace's Supplemented (TNM-FH) Insect Cell Culture Medium supplemented with 10% heat-inactivated fetal bovine serum).

Product Qualification

Frozen catalog Sf9 cells are performance tested for viability and cell growth post-recovery from cryopreservation, and are screened for mycoplasma and sterility. Master Cell Banks are screened for viruses, mycoplasma, and sterility. Species identity is confirmed by isozyme and karyotype analysis.

References

1. Smith, G.E., Ju, G., Ericson, B.L., Moshera, J., Lahm, H., Chizzonite, R. and Summers, M.D. (1985) *Proc. Natl. Acad. Sci. USA 82*, 8404.
2. Vaughn, J.L., Goodwin, R.H., Tompkins, G.J., and McCawley, P. (1977) *In Vitro 13*, 213.
3. Smith, G.E., Summers, M.D. and Fraser, M.J. (1983) *Mol. Cell. Biol. 3*, 2156.
4. Luckow, V.A. and Summers, M.D. (1988) *Bio/Technology 6*, 47.
5. Godwin, G. and Whitford, W. (1993) *Focus 15*, 44.
6. Grace, T.D.C. (1962) *Nature 195*, 788.

Related Products

	Quantity	Cat. No.
Sf-900 II SFM	1 L	10902-088
Penicillin-Streptomycin	100 mL	15070-063
Grace's Insect Cell Culture Medium, Supplemented	500 mL	11605-094
Fetal Bovine Serum, Heat-Inactivated	500 mL	10082-147
Cellfectin® Reagent	1 mL	10362-010

Note: Other reagent sizes are available.

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.

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