Sf9 cells in Sf-900™ II SFM and Sf9 cells in Sf-900™ III SFM

Catalog Numbers 11496015 and 12659017

Pub. No. MAN0007364 Rev. C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ Sf9 cells in Sf-900™ II SFM (Cat. No. 11496015) and Sf9 cells in Sf-900™ III SFM (Cat. No. 12659017) are derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf-21-AE and adapted to grow in Gibco™ Sf-900™ II SFM and Sf-900™ III SFM. The cells are prepared from low passage Master Cell Bank cultures: 40–50 total passages, 10–20 serum-free for Cat. No. 11496015; and 50–60 total passages, 25–40 serum-free for Cat. No. 12659017.

Sf-900™ II SFM and Sf-900™ III SFM are serum-free, protein-free insect cell culture media optimized for the growth and maintenance of *Spodoptera frugiperda* cells and for large-scale production of recombinant proteins expressed using the baculovirus expression vector system (BEVS).

Contents and storage

Product	Cat. No.	Amount	Storage
Sf9 cells in Sf-900™ II SFM	11496015	1 vial[1]	–200°C to –125°C, Liquid nitrogen vapor-phase
Sf9 cells in Sf-900™ III SFM	12659017	1 vial[1]	–200°C to –125°C, Liquid nitrogen vapor-phase

[1] 1 vial contains ≥1.5 × 10^7 cells in Sf-900™ II SFM or Sf-900™ III SFM with 7.5% DMSO.

Important guidelines

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.
- Prior to starting experiments, ensure you have established cells and have frozen stocks on hand. Upon receipt, grow and freeze multiple vials of cells to ensure that you have an adequate supply of early-passage cells.
- Sf-900™ II SFM and Sf-900™ III SFM are complete 1X liquid media containing L-glutamine. Supplementation with L-glutamine or a surfactant is not required.

Prepare medium

Antibiotics are not recommended; however 2.5–5 mL/L of 5000 U/5000 µg Penicillin-Streptomycin may be used when required.

Culture conditions

Media: Sf-900™ II SFM or Sf-900™ III SFM

Cell lines: Sf9 cells

Culture type: Suspension or adherent

Culture vessels: Shake flasks, spinner flasks, or T-flasks

Temperature range: 26°C to 28°C

Incubator atmosphere: Non-humidified, air regulated, non-CO_2 atmosphere. Ensure proper gas exchange and minimize exposure of cultures to light.

Guidelines and procedures for suspension Sf9 cultures

Thaw Sf9 cells, then seed in suspension cultures

1. Obtain a sterile, disposable, non-vented 125-mL polycarbonate Erlenmeyer shake flask.

   Note: Do not thaw cells in a flask larger than 125 mL, as the cell density and aeration will not be ideal.

2. Aseptically transfer 25 mL of Sf-900™ II SFM or Sf-900™ III SFM to the 125-mL shake flask. Place the flask in an incubator at 26°C to 28°C , or allow the medium in the flask to come to room temperature. Protect the medium from light exposure at all times.

3. Quickly thaw a frozen vial of Sf9 cells in a 37°C water bath until only a small frozen piece remains in the vial (~1–2 minutes). Do not submerge the entire vial under water.

4. Just before the cells have completely thawed, spray the vial with 70% isopropanol or 70% ethanol to decontaminate.

5. Gently triturate and aseptically transfer the entire contents of the vial to the flask containing 25 mL medium.

6. Place the 125-mL shake flask in an incubator (set at 26°C to 28°C without CO_2 and without humidification) on an orbital shaker platform (set at 125–150 RPM™). Loosen the shake flask cap to enable gas exchange. Protect the culture from light exposure.

7. Leave the Sf9 flask undisturbed for 3–4 days. Beginning on day 3 after thawing the cells, follow the guidelines in Table 1 and Table 2 to passage the suspended Sf9 cell cultures.

Table 1 Day 3 after thawing cells

If cell viability is	And viable cell density is	Take this action	Culture conditions
≥ 80%	≥ 2×10^6 cells / mL	Passage cells.	Seed a new 125-mL shake flask at 4×10^5 viable cells/mL in a 30–50 mL volume.
	< 2×10^6 cells/mL	Culture for one more day. Count again on day 4 (see Table 2).	—
< 80%	≥ 2×10^6 cells/mL	Pellet cells at 130 × *g* for 3 minutes.	Resuspend cells at 4 × 10^5 viable cells/mL in a 30–50 mL volume. Transfer to a new 125 mL shake flask.

Table 2 Day 4 after thawing cells *if* cells were not passaged on day 3

If cell viability is	And viable cell density is	Take this action	Culture conditions
≥ 80%	≥ 2×10^6 cells/mL	Passage cells.	Seed a new 125-mL shake flask at 4×10^5 viable cells/mL in a 30–50 mL volume.
	< 2×10^6 cells/mL	Pellet cells at 130 × *g* for 3 minutes.	Resuspend cells in 25 mL of Sf-900™ II SFM or Sf-900™ III SFM medium, then transfer to a new shake flask. Passage the cells when the cell density is ≥2×10^6 viable cells/mL (1–2 days).
< 80%	≥ 2×10^6 cells/mL	Pellet cells at 130 × *g* for 3 minutes.	Resuspend cells at 4 × 10^5 viable cells/mL in a 30–50 mL volume. Transfer to a new 125-mL shake flask.
	< 2×10^6 cells/mL	Thaw a new vial of cells, or contact Technical Support.	Follow the protocol as described (see "Thaw Sf9 cells, then seed in suspension cultures" on page 2).

Guidelines for cryopreserving Sf9 cells

Cells can be cryopreserved once the culture maintains >90% viability, achieves ≥ 2×10^6 viable cells/mL at subculture, and has gone through a minimum of 3 post-thaw passages. See "Cryopreserve Sf9 cells" on page 4.

Guidelines for seeding density

IMPORTANT! Allow cells to achieve a minimum of 2×10^6 viable cells/mL before passaging to maintain cells in mid-log growth. We recommend that you maintain stock cell counts at 2×10^6–4×10^6 viable cells/mL at passage. Over time, cell doubling times may decrease and you may need to adjust seeding densities in order to maintain cells in mid-log growth. To maintain stock cultures within the 2×10^6–4×10^6 viable cells/mL range, adjust the seeding densities according to the long-term guidelines in Table 3.

Table 3 Recommended seeding densities for initial and long-term suspension stock maintenance

Passage schedule	Sf9 seeding density	
	Initial	Long-term
3-day passage (for example, on Monday for a Monday/Thursday schedule)	4.0×10^5 viable cells/mL	3.25×10^5–4.0×10^5 viable cells/mL
4-day passage (for example, on Thursday for a Monday/Thursday schedule)	3.0×10^5 viable cells/mL	2.75×10^5–3.0×10^5 viable cells/mL

Guidelines for culture volumes

We recommend that you use the following culture volumes to help provide proper aeration for cells in order to maintain robust cell growth with doubling times in the range of 24–30 hours:

- 30–50 mL in a non-vented 125-mL shake flask
- 75–100 mL in a non-vented 250-mL shake flask
- 125–160 mL in a non-vented 500-mL shake flask

Generate a growth curve to determine ideal cell density

We recommend that you generate a growth curve using your standard culture conditions to determine the ideal cell density range for routine maintenance. Generally, cell stocks should be subcultured when they have achieved 2–4 × 10^6 viable cells/mL in order to maintain mid-log growth. Subculturing stocks when they are <2 × 106 viable cells/mL, or >4.5 × 10^6 viable cells/mL, may cause cells to lag. The information gathered from generating a growth curve can also be used to determine cell doubling times and/or growth phase under the specific culture conditions.

The following protocol is designed for insect cells grown in suspension culture using shake flasks or spinner flasks. The protocol requires performing daily viable cell counts for 7–9 consecutive days. *Before you generate a growth curve, the stock culture should be >90% viable and achieve a density ≥2 × 10^6 viable cells/mL at* subculture.

1. Remove stock culture from the incubator, and then determine cell density and viability using an automated cell counter or a hemocytometer.

2. Passage stock cells into three separate flasks at a seeding density of 3 × 10^5 viable cells/mL. Two of the flasks are used to generate growth curve data, and the third is used to continue culturing stock cells. Label each flask appropriately.

3. Incubate cells in a 26°C to 28°C, non-CO_2, non-humidified incubator on a shaker apparatus (125–150 RPM™) or spinner platform. Loosen caps to enable appropriate aeration. Record the time when the cells are placed into the incubator.

4. 24 hours after placing the cells in the incubator (±1 hour), remove the two growth curve flasks from the incubator.

5. Remove a small volume of cell suspension from each flask to determine the cell density and viability. Promptly return the flasks to the 26°C to 28°C incubator. Perform a viable cell count using an automated cell counter or hemocytometer.

6. Continue performing daily viable cell counts (see steps 4 on page 3 and 5 on page 3) for 7–9 days or until the cells have achieved the peak (highest) cell density and viability has fallen below 85%.

7. Graph the mean daily viable cell count data as shown in Figure 1, and then determine the day that the peak cell density was achieved.

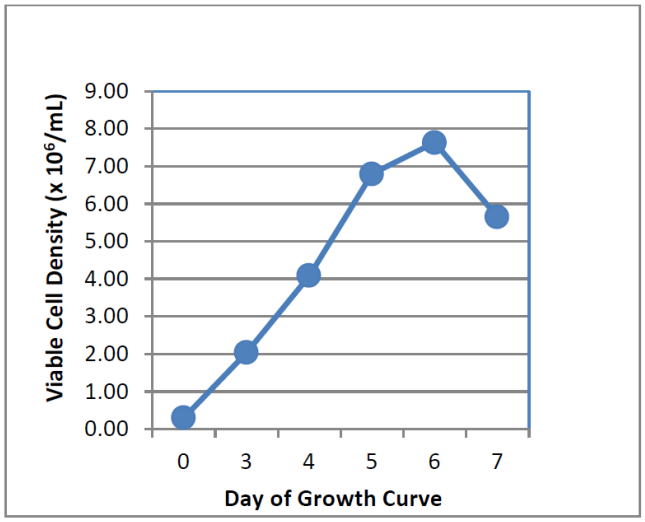

Figure 1 Example growth curve

8. Routinely passage cell stocks 2–3 days before the peak day determined using this method in order to maintain mid-log growth.

Guidelines for scaling up Sf9 cells into spinner culture

Determine and optimize the appropriate spinner or impeller speed and seeding density for your 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 on the spinner flask for proper aeration (for example, a 250-mL spinner flask should not contain >150 mL of culture).
- **Spinner or impeller speed:** Determine the optimum impeller speed for your spinner flask 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 flask walls or base.

Guidelines and procedures for adherent Sf9 cultures

Thaw Sf9 cells, then seed in adherent cultures

1. Pre-warm 40 mL Sf-900™ II SFM or Sf-900™ III SFM to room temperature in a 50-mL centrifuge tube. Protect medium from light exposure at all times.

2. Quickly thaw a frozen vial of Sf9 cells in a 37°C water bath until only a small frozen piece remains in the vial (~1–2 minutes). Do not submerge the entire vial under water.

3. Just before the cells have completely thawed, spray the vial with 70% isopropanol or 70% ethanol to decontaminate.

4. Gently resuspend cells in 10 mL of pre-warmed medium.

5. Remove a small volume of cell suspension to perform a viable cell count using an automated cell counter or hemocytometer.

6. Determine cell density and viability. Seed cells at 6×10^4 viable cells/cm^2 in appropriate tissue culture vessels based on determined viable cell density. Cells can be thawed into three T-75 flasks or ten T-25 flasks. If thawing into T-75 flasks, the total volume of medium and cell suspension should be 15–20 mL/flask. If thawing into T-25 flasks, the total volume of medium and cell suspension should be 8–10 mL/flask.

7. Place flasks into a 26°C to 28°C, non-CO_2, non-humidified incubator. Gently swirl flasks in a pattern to evenly distribute cells.

8. Observe cells for 48–72 hours post-thaw. Passage cells when confluency is ~90%.

9. Continue passaging cells following the guidelines in Table 4. Once cells have been in culture for approximately 30 passages (approximately 3 months), discard the stock culture and thaw a fresh vial of cells.

   Note: Passage cells into multiple, same-size flasks, or larger flasks if you are cryopreserving low-passage cells. We recommend that cells go through at least 3 passages post-thaw before cryopreservation.

Guidelines for passaging adherent Sf9 cultures

IMPORTANT! Passage cells when they are in the mid-log phase of growth (usually this is when cells have reached ~90% confluency).

Table 4 Seeding densities for long-term adherent stock maintenance

Passage schedule	Sf9 stock seeding density
1–2 days	8×10^4–10×10^4 cells/cm^2
2–3 days	7×10^4–8×10^4 cells/cm^2
3–4 days	6×10^4–7×10^4 cells/cm^2

Passage adherent Sf9 cells (sloughing method)

1. Pre-warm the appropriate volume of medium to room temperature. Protect medium from light at all times.

2. Remove the flask from the incubator.

3. Gently pipette the medium in the flask over the surface of the cells to dislodge the cells from the flask.

4. Remove a small volume of cell suspension from each flask to determine the cell density and viability.

5. Seed cells in Sf-900™ II SFM or Sf-900™ III SFM into desired culture vessel size as recommended in Table 4.

   Note: Cells may tightly adhere to the flask in serum-free media and require additional efforts to detach. To dislodge tightly adhering cells, shake the flask vigorously two to three times using a wrist-snapping motion.

Cryopreserve Sf9 cells

Note: Do not discontinue Sf9 stock at the time of cryopreservation. Maintain a live stock culture until a vial of the cryopreserved cells has been thawed, confirmed negative for bacterial, fungal, and mycoplasma contamination, and evaluated for proper growth and morphology.

Before cryopreserving, confirm that Sf9 stock cells cultured in Sf-900™ II SFM or Sf-900™ III SFM are >90% viable and in mid-logarithmic growth phase (2×10^6–4×10^6 viable cells/mL or at determined densities generated from the growth curve).

1. Determine the appropriate cell density for freezing using Table 5.

   Table 5 Recommended cell densities for cryopreservation

Cell densities	Volume of cells	Cryovial size	Culture conditions
1×10^7–2×10^7 viable cells/mL	1–1.5 mL	2 mL	Suspension
2×10^6–5×10^6 viable cells/mL	1–1.5 mL	2 mL	Adherent

2. Prepare the required volume of freeze medium using Table 6, then sterile filter.

   Table 6 Freeze medium components and concentration

Component	Final concentration
Sf-900™ II SFM or Sf-900™ III SFM conditioned medium	46.25%
Fresh Sf-900™ II SFM or Sf-900™ III SFM without antibiotics	46.25%
Dimethyl Sulfoxide (DMSO) cryoprotectant	7.5%

3. For best results, cryopreserve Sf9 cells using a controlled rate freezing device (for example, CryoMed™ Freezer or Mr. Frosty Nalgene™ Cryo 1°C Freezing Container) following the manufacturer's directions.

4. Store frozen Sf9 cryovials in the vapor phase of a liquid nitrogen freezer (–200°C to –125°C) the next day for long term storage.

   IMPORTANT! Do not store cryovials at –80°C for long-term storage; cell viability will be compromised.

Guidelines for transfecting Sf9 cells

For optimal results, we recommend using Cellfectin™ II Reagent for transfection. Refer to the user guide accompanying the product for instructions. If you use Cellfectin™ II Reagent, you can transfect cells directly in Sf-900™ II SFM. Other transfection reagents are suitable.

Related products

Product	Cat. No.
Sf-900™ II SFM	10902088, 10902096, 10902104
Sf-900™ III SFM	12658001, 12658019, 12658027, 12658035
Sf21 cells in Sf-900™ II SFM	11497013
Sf21 cells in Sf-900™ III SFM	12682019
Grace's Insect Medium, Supplemented	11605102, 11605094
Certified FBS, Heat Inactivated, US	10082147, A3840001, A3840002
Penicillin-Streptomycin, Liquid	15070063
Cellfectin™ II Reagent	10362100
Invitrogen™ BaculoDirect™ C-Term Expression Kit	12562013
Invitrogen™ BaculoDirect™ C-Term Transfection Kit	12562039
Invitrogen™ Bac-N-Blue™ Transfection Kit	K85501
Bac-to-Bac™ Baculovirus Expression System	10359016
Bac-to-Bac™ Vector Kit	10360014
Trypan Blue Stain	15250061
Invitrogen™ Countess™ 3 Automated Cell Counter	AMQAX2000

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
C.0	03 February 2022	• Product name and description were updated throughout the manual. • Product names and catalog numbers were updated in Related products.
B.0	16 February 2020	Converted to CCMS. Rebranded. Updated contents to state liquid nitrogen vapor phase. Added the Important guidelines for thawing and storing cells topic.