# *Sf-900™ II SFM*

Pub. No. MAN0007285 Rev. 2.0

**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

Sf-900™ II SFM is a complete, serum-free, protein-free, ready-to-use insect cell culture medium developed for high cell-density growth and high-level recombinant protein expression using the Baculovirus Expression Vector System (BEVS). Sf-900™ II SFM has optimized amino acid, carbohydrate, vitamin, and lipid components, as well as a biologically active raw material providing significant improvement in cell growth, virus production, and recombinant protein expression over other serum-free or serum-supplemented media. Sf-900™ II SFM supports long term growth (>20 passages) of *Spodoptera frugiperda* (Sf9, Sf21), *Trichoplusia ni* (Tn-368), and *Lymantia dispar* (Ld) cells in both suspension and monolayer culture.

## Contents and storage

| Product[1] | Cat. No. | Amount | Storage | Shelf Life[2] |
|---|---|---|---|---|
| Sf-900™ II SFM (1x), liquid | 10902096 | 500 mL | 2°C to 8°C<br>Protect from light | 12 months |
| | 10902153 | 10 × 500 mL | | |
| | 10902088 | 1,000 mL | | |
| | 10902104 | 6 × 1,000 mL | | |
| Sf-900™ II SFM (1x), liquid | 10902161 | 5 L | | |
| | 10902179 | 10 L | | |
| | 10902187 | 20 L | | |
| Sf-900™ II SFM (1x), liquid w/o methionine or cysteine | 21012026 | 500 mL | | |

[1] Sf-900™ II SFM is a ready-to-use medium. Do not add L-Glutamine or surfactants such as Pluronic™ F-68.
[2] Shelf life is determined from Date of Manufacture.

## Culture conditions

| | |
|---|---|
| **Media** | Sf-900™ II SFM |
| **Cell line** | Sf9, Sf21, Ld, Tn-368 cells |
| **Culture type** | Suspension or Adherent |
| **Culture vessels** | Shake flask, spinner bottle, or T-flask |
| **Temperature range** | 27°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. |

## Recovery

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Transfer entire contents of the cryovial into the appropriately sized vessel so the cells are seeded at 3–5 × $10^5$ cells/mL of pre-warmed Sf-900™ II SFM.
3. Incubate at 28°C in a non-humidified, air-regulated non-$CO_2$ atmosphere, on an orbital shaker platform rotating at 120–140 rpm. Loosen flask caps to allow for gas exchange.
4. Subculture when cells reach >2 × $10^6$ viable cells/mL. We recommend subculturing cells a minimum of 3 passages before using in downstream applications.

## Subculture suspension cultures

Insect cells are sensitive to physical shearing. Ensure that impeller mechanisms rotate freely and do not contact vessel walls or base (adjust prior to autoclaving).

1. Determine viable cell density using a Countess™ Automated Cell Counter or alternative automated or manual method.
2. Seed cells at 3–5 × $10^5$ viable cells/mL in sterile culture vessels containing pre-warmed Sf-900™ II SFM (30 mL per 125-mL shake flask, 75–100 mL per 100-mL spinner bottle).
3. Incubate at 27°C to 28°C in a non-humidified, air-regulated, non-$CO_2$ atmosphere. Loosen caps to allow for gas exchange.
4. Rotate shake flask cultures on an orbital shaker platform at 120–140 rpm, set impeller stirring rate to 85–95 rpm for spinner bottles (optimum impeller speed must be empirically determined for each spinner apparatus for robust cell growth and viability).
5. Subculture cells when viable cell density reaches >2 × $10^6$ viable cells/mL (approximately twice a week) into clean, sterile flask(s) with fresh pre-warmed Sf-900™ II SFM.

**Note:** If cell debris is observed, gently centrifuge the cell suspension at 100 × *g* for 5–10 minutes and resuspend the cell pellet in fresh Sf-900™ II SFM to reduce accumulation of cell debris and metabolic waste by-products.

**Note:** We recommend thawing a fresh low-passage vial of cells every 3 months or 30 passages.

## Subculture monolayer cultures

1. Observe cell monolayer to ensure 80–90% confluence. Aspirate medium and floating cells from a confluent monolayer.
2. Add 4 mL (per 25 $cm^2$) of pre-warmed Sf-900™ II SFM to the flask and resuspend cells by repeatedly pipetting the medium across the monolayer.
3. Observe cell monolayer to ensure cell detachment from the surface of the flask. Firmly rap the side of the flask on the palm of your hand or a hard flat surface if necessary.
4. Transfer entire cell suspension to a sterile conical tube; any cell clumps quickly settle to the bottom after 1–2 minutes. Pipet the clumps into a 10-mL pipette and gently break up the clumps by pressing the pipette tip against the bottom of the tube. Gently expell the cells back into the medium and repeat if necessary to break up remaining clumps. Pipetting too harshly will decrease cell viability due to sensitivity of cells to shear force.
5. Determine viable cell density using a Countess™ Automated Cell Counter.
6. Inoculate 2–5 × $10^4$ viable cells/$cm^2$ into new culture flasks containing pre-warmed Sf-900™ II SFM (5 mL/25 $cm^2$ ).
7. Incubate at 27°C to 28°C in a non-humidified, air-regulated, non-$CO_2$ atmosphere. Loosen caps to allow for gas exchange.
8. Three days post-plating, aspirate medium from the cell monolayer and re-feed the culture with an equal volume of fresh medium gently added to the side of the flask.

**Note:** Sf9 cells are not anchorage dependent and may be transferred between monolayer and spinner/shaker culture repeatedly without noticeable change in viability, morphology, or growth rate.

## Adapt cells to Sf-900™ II SFM

It is critical that cell viability be ≥90% and growth rate be in mid-logarithmic phase prior to initiating adaptation procedures.

### Direct adaptation

Monolayer cultures only need the culture media exchanged with prewarmed Sf-900™ II SFM as described in "Subculture monolayer cultures" on page 2.

Transfer suspension cultures into Sf-900™ II SFM using this procedure.

1. Centrifuge the cell suspension at 100 × *g* for 5–10 minutes. Aspirate and discard the supernatant.
2. Resuspend the cell pellet in pre-warmed Sf-900™ II SFM at a viable cell density of >5 × $10^5$ cells/mL and transfer to appropriate culture vessel.
3. Return to incubator and monitor cell growth.

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

### Sequential adaptation

Follow the procedures for subculture of suspension or monolayer cultures with the following modifications.

1. During the adaptation procedure use a seeding density of >5 × $10^5$ viable cells/mL.
2. Subculture cells into stepwise increasing ratios of Sf-900™ II SFM to original medium with each subsequent passage (25:75, 50:50, 75:25, 90:10 followed by 100% Sf-900™ II SFM). Multiple passages may be needed at each step.

After several passages in 100% Sf-900™ II SFM, the viable cell count should exceed 2–4 × $10^6$ cells/mL with a viability exceeding 85% within 4–6 days of culture.

### Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of greater than 1 × $10^7$ cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% Sf-900™ II SFM (50:50 ratio of fresh to conditioned media) + 7.5% DMSO on the day of intended use. Store at 4°C until use.
4. Centrifuge cell suspension at 100 × *g* for 5–10 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this cell suspension into cryovials according to the manufacturer's specifications.
6. Cryopreserve in an automated or manual controlled-rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen, (vapor-phase) storage at –200°C to –125°C.

## Related products

| Product | Cat. No. |
|---|---|
| Sf9 Cells Adapted in Sf-900™ II SFM | 11496015 |
| Sf21 Cells Adapted in Sf-900™ II SFM | 11497013 |
| BaculoDirect™ C-Term Expression Kit | 11496015 |
| BaculoDirect™ C-Term Transfection Kit | 11497013 |
| Bac-N-Blue™ Transfection Kit | K85501 |
| Bac-to-Bac™ Baculovirus Expression System | 10359016 |
| Bac-to-Bac™ Vector Kit | 10360014 |
| Countess™ 3 FL Automated Cell Counter | AMQAF2000 |

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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

**Revision history:** Pub. No. MAN0007285

| Revision | Date | Description |
|---|---|---|
| 2.0 | 30 August 2022 | Updating user guide to current regulatory classification, formatting standards, and style. |
| 1.0 | 13 June 2014 | New user guide for Sf-900™ II SFM medium. |

The information in this guide is subject to change without notice.