

Sf-900™ II SFM

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 *Lymantria dispar* (Ld) cells in both suspension and monolayer culture.

Product	Catalog No.	Amount	Storage	Shelf life*
Sf-900™ II SFM (1X), liquid	10902-096 10902-153 10902-088 10902-104	500 mL 10 × 500 mL 1000 mL 6 × 1000 mL	2°C to 8°C; Protect from light	12 months
Sf-900™ II SFM (1X), liquid, Universal Bag	10902-161 10902-179 10902-187	5 L 10 L 20 L	2°C to 8°C; Protect from light	12 months
Sf-900™ II SFM (1X), liquid w/out methionine or cysteine	21012-026	500 mL	2°C to 8°C; Protect from light	12 months

* Shelf life duration is determined from Date of Manufacture.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

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

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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-CO₂ 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 that the cells are seeded at 3–5 × 10⁵ cells/mL of pre-warmed Sf-900™ II SFM.
3. Incubate at 28°C in a non-humidified, air regulated non-CO₂ 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 × 10⁶ 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 × 10⁵ 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-CO₂ 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 × 10⁶ viable cells/mL (about 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 cm²) 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.

- 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 and gently expell the cells back into the medium, repeat if necessary to break up remaining clumps. Pipetting too harshly will decrease cell viability due to sensitivity of cells to shear force.
- Determine viable cell density using a Countess® Automated Cell Counter.
- Inoculate $2\text{--}5 \times 10^4$ viable cells/cm² into new culture flasks containing pre-warmed Sf-900™ II SFM (5 mL/25 cm²).
- Incubate at 27°C to 28°C in a non-humidified, air regulated non-CO₂ atmosphere. Loosen caps to allow for gas exchange.
- 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 $\geq 90\%$ and the growth rate be in mid-logarithmic phase prior to initiating adaptation procedures.

Direct adaptation

Monolayer cultures need only have the culture media exchanged with prewarmed Sf-900™ II SFM as described in **Subculture monolayer cultures**.

Transfer suspension cultures into Sf-900™ II SFM as follows:

- Centrifuge the cell suspension at $100 \times g$ for 5–10 minutes. Aspirate and discard the supernatant.
- Resuspend the cell pellet in pre-warmed Sf-900™ II SFM at a viable cell density of $>5 \times 10^5$ cells/mL and transfer to appropriate culture vessel.
- 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.

- During the adaptation procedure use a seeding density of $>5 \times 10^5$ viable cells/mL.
- 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 at each step may be needed.

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

Cryopreservation

- Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability $>90\%$. Reserve the conditioned medium to prepare cryopreservation medium.

- Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of $>1 \times 10^7$ cells/mL.
- 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 day of intended use, store at 4°C until use.
- Centrifuge cell suspension at $100 \times g$ for 5–10 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
- Dispense aliquots of this cell suspension into cryovials according to the manufacturer's specifications.
- Cryopreserve in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
- Transfer frozen cells to liquid nitrogen, (vapor phase) storage at -200°C to -125°C .

Related products

Product	Catalog no.
Sf9 Cells Adapted in Sf-900™ II SFM	12659
Sf21 Cells Adapted in Sf-900™ II SFM	12682
BaculoDirect™ N-Term Expression Kit	12562-054
BaculoDirect™ N-Term Transfection Kit	12562-062
BaculoDirect™ C-Term Expression Kit	12562-013
BaculoDirect™ C-Term Transfection Kit	12562-039
Bac-N-Blue™ Transfection Kit	K855-01
Bac-to-Bac® Baculovirus Expression System	10359
Bac-to-Bac® Vector Kit	10360
Countess® Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

				
Temperature Limitation	Manufacturer	Batch code	Use By:	Catalog number
				
Caution, consult accompanying documents	Consult instructions for use	Keep away from light	Sterilized using aseptic processing techniques	

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 found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support. For further assistance, email techsupport@lifetech.com

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Pluronic is a trademark of BASF Corporation. ©2014 Thermo Fisher Scientific Inc. All rights reserved.

DISCLAIMER - LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

www.lifetechnologies.com

life
technologies™