gibco
by life technologies

Sf-900™ III SFM

Description

Sf-900™ III SFM is a complete serum-free, protein-free, and animal origin-free (AOF), ready-to-use medium developed for the growth of *Spodoptera frugiperda* (Sf9, Sf21) cells for expression of recombinant proteins in suspension culture using Baculovirus Expression Vector Systems (BEVS). Sf-900™ III SFM contains L-glutamine, Pluronic® F-68 and a reduced level of hydrolysate compared to Sf-900™ II SFM.

Product	Catalog no.	Amount	Storage	Shelf life*
Sf-900™ III SFM (1X), liquid	12658-019 12658-027 12658-035 12658-001	500 mL 1000 mL 10 L, Universal Bag 20 L, Universal Bag	2°C to 8°C; Protect from light	18 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™ III SFM is a complete, ready to use medium. Do not add L-Glutamine or surfactants such as Pluronic® F-68.

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

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™ III SFM

Cell line(s): Sf9, Sf21 cells

Culture type: Suspension or Adherent

Recommended culture vessels: Shake flask or spinner bottle

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 vial of cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a 125-mL shake flask containing 28.5 mL of pre-warmed Sf-900™ III SFM.
3. Incubate at 27°C to 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.

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.
2. Seed cells at 3–5 × 10^5 viable cells/mL in sterile culture vessels containing pre-warmed Sf-900™ III 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. For spinner cultures set impeller stirring rate to 85–95 rpm (rpm may vary with impeller design). Loosen side arm caps to allow for gas exchange.

   Note: Impeller paddles should extend slightly above the medium surface to provide additional aeration to the cultures.
5. Subculture cells when viable cell density reaches >2 × 10^6 viable cells/mL (about twice a week) into clean, sterile flask(s) with fresh pre-warmed Sf-900™ III SFM.

Note: To reduce accumulation of cell debris and metabolic waste by-products in suspension cultures, gently centrifuge the cell suspension at 100 × *g* for 5–10 minutes and resuspend pellet in fresh Sf-900™ III SFM once every 3 weeks.

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

Subculture monolayer cultures

1. Observe cell monolayer using an inverted microscope to ensure confluence. Aspirate, and discard, medium and floating cells from a confluent monolayer.
2. Add 4 mL (per 25 cm^2) pre-warmed Sf-900™ III SFM to the flask and resuspend cells by repeatedly pipetting the medium across the monolayer.
3. Observe cell monolayer using an inverted microscope to ensure cell detachment from the surface of the flask. To aid cell detachment it may be necessary to firmly rap the side of the flask on the palm of your hand or a hard flat surface.
4. Transfer entire cell suspension from the flask to a sterile conical tube, cap securely, and vortex (~15 seconds).
5. Determine viable cell density using a Countess® Automated Cell Counter.
6. Inoculate 1–1.6 × 10^6 cells (per 25 cm^2) into new culture flasks containing pre-warmed Sf-900™ III SFM (5 mL per 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 and return to the incubator.

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

Adapt Sf9 and Sf21 cells to Sf-900™ III SFM

We recommend using sequential adaptation when adapting cells to Sf-900™ III SFM. It is critical that cell viability be at least 90% and the growth rate be in mid-logarithmic phase prior to initiating adaptation procedures.

1. Subculture Sf9 or Sf21 cells into a 25:75 ratio of Sf-900™ III SFM to the original media. During the adaptation procedure use a seeding density of 6–8 × 10^5 viable cells/mL.
2. Incubate at 27°C to 28°C in a non-humidified, non-CO_2 atmosphere on an orbital shaker platform rotating at 120–140 rpm. Loosen caps to allow for gas exchange.
3. Subculture when the viable cell count is >2 × 10^6 cells/mL (4–6 days post plating) by passaging cells into a 50:50 ratio of Sf-900 III SFM to original medium.
4. Repeat step 3, increasing stepwise the ratio of Sf-900™ III SFM to original medium (75:25 followed by 90:10) until the cells are transferred into 100% Sf-900™ III SFM. Multiple passages at each step may be needed.

After several passages in 100% Sf-900™ III SFM, the viable cell count should exceed 4 × 10^6 cells/mL with a viability exceeding 85% within 4–6 days of culture. At this stage the culture is considered to be adapted to Sf-900™ III SFM. The seeding density may be reduced to 3–5 × 10^5 viable cells/mL during the final stages of adaptation.

Cryopreservation

1. Prepare the desired quantity of cells in either a spinner or shaker culture, 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 >1 × 10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium of Sf-900™ III SFM (50:50 ratio of fresh to conditioned media) +7.5% DMSO on day of intended use, store at 4°C until use.
4. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2-mL cryovial).
6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen, we recommend (vapor phase) storage at –200°C to –125°C.

Related products

Product	Catalog no.
Sf9 Cells Adapted in Sf-900™ III SFM	12659
Sf21 Cells Adapted in Sf-900™ III SFM	12682
Sf-900™ II SFM, liquid	10902
Penicillin-Streptomycin, liquid	15070
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:

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

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.

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**.

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**