

Sf9 (*Spodoptera frugiperda*) Insect Cells

INSTRUCTIONS FOR USE

Product Description

The Sf9 insect cell line is a clonal isolate derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf-21-AE. Sf9 cells are adapted for suspension culture in ESF 921 or ESF AF and are available as a frozen vial or suspension culture.

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

Product	Catalog Number	Amount	Storage
Sf9 cells adapted in ESF 921, frozen vial	94-001F	50 million cells per vial	Thaw immediately or LN ₂
Sf9 cells adapted in ESF 921, suspension culture	94-001S	50 million cells in 50 mL media	Culture immediately
Sf9 cells adapted in ESF AF, frozen vial	94-006F	50 million cells per vial	Thaw immediately or LN ₂
Sf9 cells adapted in ESF AF, suspension culture	94-006S	50 million cells in 50 mL media	Culture immediately

Important Information

ESF 921 and ESF AF are 1X complete, ready to use media. Do not add L-Glutamine or surfactants such as Pluronic® F-68. Antibiotics are not recommended; however, Penicillin-Streptomycin or Gentamicin may be used when required.

Safety Information

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

Culture Conditions

Media: ESF 921 or ESF AF

Cell Line(s): Sf9

Culture Type: Suspension or adherent

Recommended Culture Vessels: Shake flask or T-flask

Temperature Range: 27°C to 28°C

Incubator Atmosphere: Non-humidified, non-CO₂ atmosphere. Ensure proper gas exchange and minimize exposure of cultures to light.

Receiving Frozen Cells

Insect cells are frozen in ESF 921 or ESF AF with 10% DMSO. There are 50 x 10⁶ cells per vial.

1. Prepare for thawing cells by placing 50 mL of room temperature ESF 921 or ESF AF into a 125 mL Erlenmeyer shake flask.
2. Thaw frozen cells rapidly by swirling vial in a 37°C water bath. Thaw until a small amount of ice remains. Do not leave vial unattended.
3. Sanitize outer surface of vial with 70% alcohol. Transfer contents of vial to culture flask using a 2 mL pipette. Do not pour.
4. Incubate overnight at 27°C in a non-humidified, non-CO₂ atmosphere incubator. Loosen caps (both solid and vented) to allow for gas exchange. Allow the cells to achieve a density of at least 4 x 10⁶ cells per mL before passaging.
5. Split cells at a density of 1 x 10⁶ cells per mL for the first week after thaw. Follow directions for suspension culture thereafter.

Receiving Suspension Cultures

Insect cells are packaged in 125 mL Erlenmeyer shake flasks. There are 50 x 10⁶ cells in 50 mL of media per flask.

1. Remove parafilm and loosen cap for good aeration. Determine cell count and viability.
2. Place flask in a shaker incubator at 130-135 rpm at 27°C. Cells should start doubling a day after receipt. It is not unusual for the cell count to remain the same the first 24 hours after receipt. Allow the cells to reach a density of 4 x 10⁶ cells per mL before passaging.

Suspension Cell Culture

Sf9			
Max Density	>15x10 ⁶ /mL	Split Density	6-8x10 ⁶ /mL
Seed Density	0.75-1x10 ⁶ /mL	Split Frequency	2x/week

Passage the cells twice a week on a Mon/Thurs or Tues/Fri schedule. Repeatedly allowing the cells to reach maximum density may change the growth kinetics of the culture. Split the cells while still in mid-log phase growth.

Note: It is recommended that a growth curve be determined using the user's standard culturing conditions. This will allow for determination of mid-log phase growth.

1. Determine viable cell count.
2. Seed shake flask at a density shown above. Use 30-50 mL for a 125 mL Erlenmeyer shake flask.
3. Incubate at 27°C in a non-humidified, non-CO₂ atmosphere incubator. Shake cultures on an orbital shaker platform at 130-135 rpm. Loosen caps (both solid and vented) to allow for gas exchange.
4. Passage when viable cells density reaches 6-8 x 10⁶ cells per mL.
5. It is recommended to thaw a new vial of cells every 3 months. Cultures may be maintained for longer but increase the risk of accumulating environmental stresses that can impact the growth and performance characteristics of the culture.

Monolayer Cell Culture

1. Observe cell monolayer using an inverted microscope to assess confluence. Remove media and any floating cells using a sterile pipette or by aspiration.
2. Add 4 mL (per 25 cm²) ESF 921 or ESF AF to the flask and resuspend the cells by repeatedly pipetting the medium across the monolayer. It may be necessary to aid cell detachment by tapping the side of the flask against a hard surface.
3. Determine the viable cell density of the cell suspension.
4. Inoculate 0.5-1 x 10⁶ cells (per 25 cm²) into new culture flasks containing room temperature ESF 921 or ESF AF (5 mL per 25 cm²).
5. Incubate at 27°C in a humidified, non-CO₂ atmosphere incubator or chamber. Ensure air exchange by loosening caps. Vented caps are recommended.

Cryopreservation

1. Freezing medium is sterile filtered 90% ESF 921 or ESF AF plus 10% DMSO. 0.15 M trehalose may be added. Store and use at 4°C.
2. Prepare the desired quantity of cells, harvesting in mid-log growth with viability >90%.
3. Determine the viable cell density and calculate the required volume of freezing medium to give a final cell density between 25-50 x 10⁶ cells per mL.
4. Harvest the cells by centrifugation at 1000 rpm for 5 minutes. Resuspend the cells in the pre-determined volume of 4°C freezing medium.
5. Dispense 1 mL aliquots of suspension into cryovials.
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.

Limited Product Warranty








Expression Systems LLC warrants that this product meets its specifications, as stated in our product brochures and certificates. This warranty lasts from the time we deliver the consumable until either the consumable's shelf life, when the product has been handled and stored in accordance with this IFU.

For technical assistance or documentation, such as Certificates of Analysis or Safety Data Sheets, email support@expressionsystems.com

Related Products

Product	Catalog Number
ESF 921	96-001
ESF AF	99-300
Production Boost Additive	95-006
Adapted Sf21 Cells	94-003 / 94-010
Adapted Tni Cells	94-002 / 94-011
Adapted S2 Cells	94-005 / 94-012
BestBac™ Linearized DNA	91-001 / 91-002
Transfection Medium	95-020

Legend of Labeling Symbols

Symbol	Interpretation
	Catalog Number
	Lot Number
	<i>Research Use Only</i>
	Manufacturer
	Temperature Limitation
	Date of Manufacture
	Instruction for Use

Important Licensing Information

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

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