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 flasks or spinner bottle
Temperature Range: 27°C to 28°C
Incubator Atmosphere: Non-humidified, non-CO_2 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 10^6 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 shaking in a 37ºC water bath. Thaw vial 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 1 ml pipette. Do not pour.
4. Incubate overnight at 27ºC in a non-humidified, non-CO_2 atmosphere incubator. Loosen caps to allow for gas exchange. Allow the cells to achieve a density of at least 4 x 10^6 cells per ml before passaging.
5. Split cells at a density of 1 x 10^6 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 10^6 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 120-140 rpm at 27ºC. 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 10^6 cells per ml before passaging.

Suspension Cell Culture

	Sf9
Max Density	>15 x10^6/mL
Split Density	6-8 x 10^6/mL
Seed Density	0.75-1 x 10^6/mL
Split Frequency	2-3x/week

It is recommended to passage the cells three days a week on a Mon/Wed/Fri schedule or twice a week on a Mon/Thurs or Tues/Fri schedule. It is not advised to repeatedly allow the cells to reach maximum density as the growth kinetics of the culture may change. Try to 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, 50-75 mL for 100 mL spinner bottle.
3. Incubate at 27°C in a non-humidified, non-CO_2 atmosphere incubator. Rotate shake flask cultures on an orbital shaker platform at 120-140 rpm. Loosen caps to allow for gas exchange. 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.

4. Passage when viable cells density reaches 6-8 x 10^6 cells per mL.
5. It is recommended to thaw a new vial of cells every 3 months. Cultures may be maintained for a longer time period 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 ensure confluence. Remove media and any floating cells using a sterile pipette or by aspiration.
2. Add 4 mL (per 25 cm^2) ESF 921 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 10^6 cells (per 25 cm^2) into new culture flasks containing room temperature ESF 921 (5 mL per 25 cm^2).
5. Incubate at 27°C in a non-humidified, non-CO_2 atmosphere incubator. Loosen caps or use flasks with vented caps (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 4ºC.
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 10^6 cells per mL.
4. Harvest the cells by centrifugation at 1000 rpm for 5 minutes. Resuspend the cells in the pre-determined volume of 4ºC 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 (1ºC decrease per minute).
7. Transfer frozen cells to liquid nitrogen, we recommend vapor phase storage at -200 ºC to -125 ºC.

Related Products

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

Legend of Labeling Symbols

Symbol	Interpretation
REF	Catalog Number
LOT	Lot Number
RUO	*Research Use Only*
	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.

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

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