Expression Systems
An Advancion Company

Sf9 in ESF AdvanCD™ Insect Cells
INSTRUCTIONS FOR USE

Product Description

The Sf9 in ESF AdvanCD™ Insect Cell Line is a clonally-derived Sf9 cell line grown in suspension culture and descends from an isolate originally derived from ovarian tissue of *Spodoptera frugiperda (Sf) pupae*. The parental line, IPLB-SF-21 (renamed IPLB-SF-21 AE after adaptation into TC-100 medium), was established by J. Vaughn at the Insect Pathology Lab in Beltsville, Maryland, USA in the late 1960's. A subclone, designated Sf9, was established in 1983 by G. Smith and C. Cherry, and subsequently deposited with ATCC. A vial of ATCC CRL-1711 was obtained by Expression Systems and the Sf9 in AdvanCD cell line was cloned from lot 70020337.

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

Product	Catalog Number	Amount	Storage
Sf9 cells in ESF AdvanCD™, frozen vial	94-030	50 million cells per vial	Thaw immediately or LN_2

Important Information

ESF AdvanCD is 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 AdvanCD

Cell Line(s): Sf9

Culture Type: Suspension

Recommended Culture Vessels: Shake flasks

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 AdvanCD with 10% DMSO. There are 50 x 10^6 cells per vial. It is always recommended to create a small cell bank 2-3 passages after thaw to ensure availability of healthy cells.

1. Prepare for thawing cells by placing 50 mL of room temperature ESF AdvanCD into a 125 mL Erlenmeyer shake flask.
2. Thaw frozen cells rapidly by swirling 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 2 mL pipette. Do not pour.
4. Incubate overnight at 27ºC in a non-humidified, non-CO_2 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 10^6 cells per mL before passaging.

Suspension Cell Culture

Sf9 in ESF AdvanCD			
Max Density	>30x10^6/mL	Split Density	4-18x10^6/mL
Minimum Seed Density	0.5x10^6/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-CO_2 atmosphere incubator. Shake cultures on an orbital shaker platform at 130-135 rpm. Loosen caps on non-vented caps to allow for gas exchange.
4. Passage when viable cells density reaches 4-18 x 10^6 cells per mL.
5. Recommended seed densities are 0.75x10^6/mL for 3 day passages (i.e. Mon-Thur) and 0.5x10^6/mL for 4 day passages (i.e. Thurs-Mon).
6. 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.

Cryopreservation

1. Freezing medium is sterile filtered 90% ESF AdvanCD 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 >95%.
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.

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4. Harvest the cells by centrifugation at 300-400 x g 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.

For Research Use Only

This product is 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. Product is not to be used in human, animal or diagnostic applications or disclosed to any third party without prior written consent.

Related Products

Product	Catalog Number
ESF AdvanCD™	54-018
BestBac™ Linearized DNA	91-001 or 91-002
Virus Stabilization Additive (VSA)	95-010
Transfection Medium	95-020

Legend of Labeling Symbols

Symbol	Interpretation
	Catalog Number
	Lot Number
	*Research Use Only*
	Manufacturer
	Temperature Limitation
	Date of Manufacture
	Instruction for Use

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

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