ESF AdvanCD™

INSECT CELL CULTURE MEDIUM CHEMICALLY DEFINED INSTRUCTIONS FOR USE

Product Description

ESF AdvanCD™ Insect Cell Culture Medium is a complete chemically defined, serum-free, protein-free medium developed for robust cell growth, protein expression and baculovirus vector production for a wide range of insect cells including Sf9, Sf21, and Tni. ESF AdvanCD contains Alanyl-Glutamine, L- Glutamine and Kolliphor® P188 (Pluronic® F-68).

Virus Stabilization Additive (VSA) is an animal-free and protein-free stabilizer of recombinant baculovirus stocks produced in ESF AdvanCD. When stored at 4°C, virus stocks are less prone to aggregation when VSA is added.

For Research Use or Further Manufacturing. Not for diagnostic use or direct administration into humans or animals.

Product	Catalog Number	Volume	Storage	Recommended Use By Date
ESF AdvanCD™ (1X), liquid	54-018-01 Custom	1 Liter Inquire for Custom Fill Volumes	2°C to 8°C, protected from light	12 months from Date of Manufacture
Virus Stabilization Additive	95-010-020 95-010-100	20 mL 100 mL	2°C to 8°C, protected from light	12 months from Date of Manufacture

Important Information

ESF AdvanCD is a 1X complete, ready to use medium. 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): Sf21, Sf9, RV-Free, TniPRO, HighFive
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.

Suspension Cell Culture

	Sf9	RV-Free	TniPRO
Max Density	>30 x10^6/mL	>35 x 10^6/mL	>10 x 10^6/mL
Split Density	4-18 x 10^6/mL	4-20 x 10^6/mL	3-8 x 10^6/mL
Seed Density	0.75-1 x 10^6/mL	0.75-1 x 10^6/mL	0.3-0.5 x 10^6/mL
Split Frequency	2-3x/week	2-3x/week	2-3x/week

It is recommended to passage the cells twice a week on a Mon/Thurs (Preferred) or Tues/Fri schedule or three days a week on a Mon/Wed/Fri schedule. It is not advised to repeatedly allow the cells to reach maximum densities 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 in table. Use 30-50 mL for a 125 mL Erlenmeyer shake flask, 80-100 mL for a 250 mL flask, 300 mL for a 1L flask.
3. Incubate at 27°C in a non-humidified, non-CO_2 atmosphere incubator. Rotate shake flask cultures on an orbital shaker platform at 130-140 rpm with a throw of 25 mm. Loosen caps to allow for gas exchange.
4. Passage when viable cell density reaches range listed in the table.
5. It is recommended to thaw a new vial of cells every 3 months. Cultures may be maintained for a longer time but increase the risk of accumulating environmental stresses that can impact the growth and performance characteristics of the culture.

Monolayer Cell Culture

Adherent culture is not recommended with ESF AdvanCD.

Adaptation of Cells to ESF AdvanCD

Always generate growth curves upon adaptation of cells into a new medium.

ESF AdvanCD has been shown to support robust cell growth following direct inoculation of cells grown in competitor's medium. However, individual users' experiences may vary. If direct inoculation into ESF AdvanCD medium is unsuccessful, it is recommended to use sequential adaptation. It is critical that cell viability be at least 95% (Sf9), 93% (Tni), or 90% (Sf21), and the growth rate be in mid-logarithmic phase prior to initiating the adaptation process.

1. Passage cells into a 50:50 ratio of ESF AdvanCD to the original media. During the adaptation process, use a seeding density of 1 x 10^6 cells per mL.

2. Incubate at 27°C in a non-humidified, non-CO_2 atmosphere incubator. Rotate shake flask cultures on an orbital shaker platform at 130-140 rpm. Loosen caps to allow for gas exchange.
3. Passage when the viable cell count is 4-6 x 10^6 cells per mL (3-4 days post-split). Passage into a 75:25 ratio of ESF AdvanCD to original medium.
4. Repeat previous step, increasing the ratio of ESF AdvanCD to original medium (75:25 followed by 88:12, 95:5) until the cells are in 100% ESF AdvanCD. It may be necessary to perform multiple passages in one ratio format.
5. Insect cells may swell during adaptation. An increase of 2-3 microns is normal, an increase of 5 microns or more is suggestive of a stressed culture and the adaptation should be started again with fresh cells.

After several passages in 100% ESF AdvanCD, the viable cell count should conform to the table above with viabilities > 90%.

Virus Amplification

1. Seed culture at a density of 1-2.5x10^6 cells/mL. Culture overnight.
2. Count cells and determine the amount of virus to add for a target MOI of 0.1. Culture for 3-4 days. Best results will occur when harvesting at a viability of > 75%.
3. Remove cells by centrifugation (time and g will vary with volume, recommendation is 1000-2000xg for 10-15 minutes).
4. Transfer clarified supernatant to a 0.2 micron bottle top filter and add 10% v/v Virus Stabilization Additive (VSA). IMPORTANT: When working with high titer baculovirus stocks, VSA must be added to avoid viral aggregation.
5. Store at 4°C in the dark.

Cryopreservation

6. Freezing medium is sterile filtered 90% ESF AdvanCD plus 10% DMSO. 0.15 M trehalose may be added. Store and use at 4ºC.
7. Prepare the desired quantity of cells, harvesting in mid-log growth with viability >90%.
8. 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.
9. Harvest the cells by centrifugation at 400g for 10-15 minutes. Resuspend the cells in the pre-determined volume of 4ºC freezing medium.
10. Dispense 1 mL aliquots of suspension into cryovials.
11. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1ºC decrease per minute).
12. Transfer frozen cells to liquid nitrogen, we recommend vapor phase storage at -200 ºC to -125 ºC.

Legend of Labeling Symbols

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

Related Products

Product	Catalog Number
ESF AdvanCD™	54-018
Virus Stabilization Additive	95-010
Sf9 Cells in ESF AdvanCD	94-030
TniPRO™ Cells in ESF AdvanCD	94-031
Sf9 RV-Free Cells	inquire
BestBac™ Linearized DNA	91-001 or 91-002

Important Licensing Information

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

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

Expression Systems LLC
2537 2nd Street, Davis, CA 95618
P: (530) 747-2035
expressionsystems.com

Doc ID: 934052
Rev. No.:1