Expression Systems
An Advancion Company

ESF AdvanCD™
INSECT CELL CULTURE MEDIUM
CHEMICALLY DEFINED

QUICK START GUIDE

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 (High Five™). 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.

Important Notes:

1. Recombinant baculovirus produced in ESF AdvanCD may achieve very high titers. Baculovirus stocks with titers of $5x10^8$ infectious units per mL will tend to aggregate, with more aggregation as the titer increases. When producing high titer baculovirus stocks using ESF AdvanCD, it is recommended to utilize VSA to minimize viral aggregation and subsequent loss of titer.
2. ESF AdvanCD is incompatible with polyplex or lipoplex formation for the purposes of transfection of insect cells growing in ESF AdvanCD. It is recommended to use a product such as Transfection Medium to form the complexes and, if using an adherent method, perform the initial complex incubation with the cells.

Quick Start Guide for Adaptation of Spodoptera or Trichoplusia insect cell cultures to ESF AdvanCD

1. Start with an actively growing culture of insect cells in logarithmic growth phase.
2. Inoculate cells into 125 mL shake flask containing ESF AdvanCD to a final concentration of 1E6 viable cells/mL. Ideally there should be no more than 10% media carryover so use cell cultures that are at a density of 5E6 cells/mL or greater.
3. Passage to 1E6 cells/mL no more than 3 days post-inoculation. If cells have not grown to a density greater than 5E6 cells/mL within 3 days of inoculation, try again with a new culture or try a slower adaptation process.
4. If cells are growing well after the first week, proceed to standard passaging conditions described below.
5. Cells grow quickly once adapted to ESF AdvanCD. Do not allow Sf9 cells to exceed 20E6/mL and Tni (High Five) cells to exceed 8E6/mL.

Use of Virus Stabilization Additive

1. VSA is used to stabilize recombinant baculovirus stocks generated in ESF AdvanCD.
2. Separate cells and cell debris from rBV containing supernatant by centrifugation or filtration.
3. Filter supernatant if desired. It is recommended to filter through a 0.2-micron filter prior to storage. This is to remove cell debris that may initiate viral aggregation.
4. Add 10% volume of VSA to clarified supernatant.
5. Store at 4°C in the dark.

Transfection Protocol

1. Transfer 200 µL of transfection media to a 1-2mL sterile polypropylene tube.
2. Add 0.5 µg BestBac to the transfection media.
3. Add 2.0 µg Plasmid to the transfection media.
4. (Optional) in the case of Bacmids BestBac and Plasmid can be replaced with 1 µg of Bacmid.
5. Add 6 µL of ExpreS^2 TR (or optimized volume of reagent of choice).
6. Vortex briefly at low speed then incubate 20 minutes at RT.
7. During incubation, seed a 20mL culture of Sf9 cells in ESF AdvanCD at 0.75E6 vc/mL in a 125mL shake flask.
8. Add transfection mixture to cell culture while swirling, making sure transfection mixture does not touch wall of the cell culture flask.
9. Harvest after 4-5 days of incubation (see below for culture conditions) or if viability drops below 80%.
10. Add 10% VSA after harvesting, if filtering it is recommended to add VSA after filtration.

High Density Infection Protocol

Sf9 cells grow rapidly and achieve high densities in ESF AdvanCD cell culture medium. This characteristic can be used to maximize per liter yields of protein by infecting at higher cell densities. This strategy can be used with both high and low MOI infections although care must be taken with low MOI cultures to ensure that the cells don't outgrow the infection. Preferably expression cultures will be seeded from a stock culture of cells in logarithmic growth, ideally between 8-20E6 vc/mL, the day before infection ensuring maximum log phase cells. Alternatively, cultures can be split to the desired infection density directly.

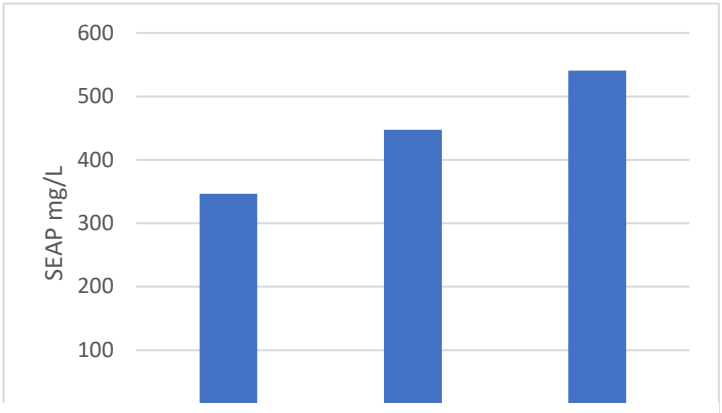
1. Seed expression cultures and allow to grow overnight.
2. Infect Cultures at desired MOI. ES recommends 0.1 for low MOI strategies and 3 for high MOI strategies. BIICs follow high MOI strategies.
3. Harvest 48 or 72 hours post infection for low MOI and 72 or 96 hours post infection for high MOI. The exact time of harvest is dependent on multiple factors and should be assessed for each protein.

	Seed Density Viable cells per mL	Infection Density Viable cells per mL	Estimated harvest density, total cells per mL	Recommended time of harvest
Low MOI (0.1)	2-2.5E6 vc/mL	5-6E6 vc/mL	7.5-9E6 c/mL	72-96 hours post infection
High MOI (3-5)	3-4E6 vc/mL*	8-11E6 vc/mL	11.5-15E6 c/mL	48-72 hours post infection

*3E6 vc/mL has been the most reproducible successful seed density for high MOI infections.

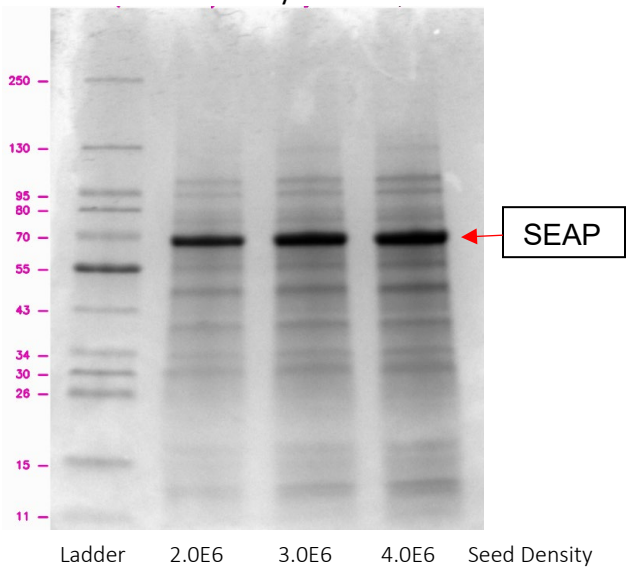
Example: SEAP Expression with Increasing Culture Density

SEAP expression using a high MOI (3) strategy with increasing seed densities. Cultures were seeded as described, allowed to grow overnight, infected at the determined densities, and then harvested 72 hours post-infection.



Seed Density	2.0E6	3.0E6	4.0E6
Infection Density	5.5E6	8.3E6	10.5E6
Harvest Density vc/mL, Viability	6.2E6, 81%	9.6E6, 84%	12.6E6, 84%
Harvest Density Total Cells/mL	7.7E6	11.5E6	15E6

Culture supernatants on SDS-PAGE stained by Coomassie



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INSTRUCTIONS FOR USE

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%.

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 >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 400g for 10-15 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 AdvanCD™	54-018
Virus Stabilization Additive	95-010
Transfection Medium	95-020
Sf9 Cells in ESF AdvanCD	94-030
Sf9 RV-Free Cells	inquire
TniPRO™ Cells in ESF AdvanCD	94-031
BestBac™ Linearized DNA	91-001 or 91-002

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