ESF AF™ INSECT CELL CULTURE MEDIUM INSTRUCTIONS FOR USE

Expression Systems
An Advancion Company

Product Description

ESF AF™ Insect Cell Culture Medium is an Animal Origin-Free formulation and is a complete 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, Tni, and Drosophila S2. ESF AF medium is comparable to ESF 921 for growth and expression. ESF AF contains L-Glutamine and Kolliphor® P188 (Pluronic® F-68). 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 AF (1X), liquid	99-300-01 99-300-10 99-300-20 99-300-50	1 Liter 10 Liter, Media Transfer Bag 20 Liter, Media Transfer Bag 50 Liter, Media Transfer Bag in Drum Inquire for Custom Fill Volumes	2°C to 8°C, protected from light	12 months from Date of Manufacture

Important Information

ESF AF 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 AF
Cell Line(s): Sf9, Sf21, Tni, S2
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.

Suspension Cell Culture

	Sf9	Sf21	Tni	S2
Max Density	>18 x10^6/mL	>5 x 10^6/mL	>8 x 10^6/mL	>100 x 10^6/mL
Split Density	6-8 x 10^6/mL	4-5 x 10^6/mL	6-7 x 10^6/mL	25-75 x 10^6/mL
Seed Density	0.75-1 x 10^6/mL	0.5-1 x 10^6/mL	0.5-1 x 10^6/mL	2-5 x 10^6/mL
Split Frequency	2-3x/week	2-3x/week	2-3x/week	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 densities as the growth kinetics of the culture may change. Try to keep the maximum cell density to mid-log phase.

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 split density described in suspension cell culture table.
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 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 10^6 cells (per 25 cm^2) into new culture flasks containing room temperature ESF AF (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).

Adaptation of Cells to ESF AF

It is recommended to use sequential adaptation when adapting cells to ESF AF medium. It is critical that cell viability be at least 95% (Sf9), 93% (Tni), 90% (Sf21) or 90% (S2), 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 AF to the original media. During the adaptation process, use a seeding density of 1 x 10^6 cells/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 120-140 rpm. Loosen caps to allow for gas exchange.

3. Passage when the viable cell count is at split density described in suspension cell culture table (3-4 days post-split). Passage into a 75:25 ratio of ESF AF to original medium.
4. Repeat previous step, increasing the ratio of ESF AF to original medium (75:25 followed by 88:12, 95:5) until the cells are in 100% ESF AF. It may be necessary to perform multiple passages in one ratio format.
5. Insect cells may swell during adaptation. An increases 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 AF, the viable cell count should conform to the table above with viabilities > 90%.

Cryopreservation

1. Freezing medium is sterile filtered 90% 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/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
Production Boost Additive	95-006
Adapted Sf9 Cells	94-001 or 94-006
Adapted Sf21 Cells	94-003 or 94-010
Adapted Tni Cells	94-002 or 94-011
BestBac™ Linearized DNA	91-001 or 91-002
Transfection Medium	95-020

Legend of Labeling Symbols

Symbol	Interpretation
	Catalog Number
	Lot Number
	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.

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