

Product Description

ESF SFM™ Mammalian Cell Culture Medium is an Animal Origin-Free formulation and is a complete serum-free medium developed for robust cell growth and protein expression for a wide range of mammalian cells including 293, CHO and hybridoma lines. ESF SFM contains L-Glutamine and Kolliphor® P188 (Pluronic® F-68). For Research Use Only. Not for use in diagnostic procedures.

| Product | Catalog Number | Volume | Storage | Shelf Life |
|----------------------|---------------------|--------------------------------------------|-------------------------------------|------------------------------------------|
| ESF SFM (1X), liquid | 98-001-01 Custom | 1 Liter Inquire for Custom Fill Volumes | 2°C to 8°C, protected from light | 12 months from Date of Manufacture |

Important Information

ESF SFM 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 SFM

Cell Line(s): 293, CHO, Hybridoma

Culture Type: Suspension or adherent

Recommended Culture Vessels: Shake flasks or spinner bottle

Temperature Range: 37°C to 38°C

Incubator Atmosphere: Humidified, 5% CO₂ atmosphere. Ensure proper gas exchange and minimize exposure of cultures to light.

Suspension Cell Culture

| | 293-ES | CHO-ES |
|-----------------|------------------------------|----------------------------------|
| Max Density | >4 x 10 ⁶ /mL | >5 x 10 ⁶ /mL |
| Split Density | 3 x 10 ⁶ /mL | 4 x 10 ⁶ /mL |
| Seed Density | 0.3 x 10 ⁶ /mL | 0.2-0.3 x 10 ⁶ /mL |
| Split Frequency | 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.

- Determine viable cell count.
- Seed shake flask at a density shown above. Use 30-40 mL for a 125 mL Erlenmeyer shake flask, 50-75 mL for 100 mL spinner bottle.
- Incubate at 37°C in a humidified, 5% CO₂ atmosphere incubator. Rotate shake flask cultures on an orbital shaker

platform at 140-150 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.

- Passage when viable cells density reaches 3-4 x 10⁶ cells/mL.
- 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

- Observe cell monolayer using an inverted microscope to ensure confluence. Remove media and any floating cells using a sterile pipette or by aspiration.
- Add 4 mL (per 25 cm²) ESF SFM 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.
- Determine the viable cell density of the cell suspension.
- Inoculate 0.5-1 x 10⁶ cells (per 25 cm²) into new culture flasks containing room temperature ESF SFM (5 mL per 25 cm²).
- Incubate at 37°C in a humidified, 5% CO₂ atmosphere incubator. Loosen caps or use flasks with vented caps (recommended).

Adaptation of Cells to ESF SFM

It is recommended to use sequential adaptation when adapting cells to ESF SFM medium. It is critical that cell viability be at least 90%, and the growth rate be in mid-logarithmic phase prior to initiating the adaptation process.

- Passage cells into a 50:50 ratio of ESF SFM to the original media. During the adaptation process, use a seeding density of 0.5-1 x 10⁶ cells/mL.
- Incubate at 37°C in a humidified, 5% CO₂ atmosphere incubator. Rotate shake flask cultures on an orbital shaker platform at 140-150 rpm. Loosen caps to allow for gas exchange.
- Passage when the viable cell count is 3-4 x 10⁶ cells/mL (3-4 days post-split). Passage into a 75:25 ratio of ESF SFM to original medium.
- Repeat previous step, increasing the ratio of ESF SFM to original medium (75:25 followed by 88:12, 95:5) until the cells are in 100% ESF SFM. It may be necessary to perform multiple passages in one ratio format.

- 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 SFM, the viable cell count should be consistently at viabilities > 90%.








Cryopreservation

- Freezing medium is sterile filtered 90% ESF SFM plus 10% DMSO. 0.15 M trehalose may be added. Store and use at 4°C.
- Prepare the desired quantity of cells, harvesting in mid-log growth with viability >90%.
- Determine the viable cell density and calculate the required volume of freezing medium to give a final cell density between 5-20 x 10⁶ cells/mL.
- Harvest the cells by centrifugation at 1000 rpm for 5 minutes. Resuspend the cells in the pre-determined volume of 4°C freezing medium.
- Dispense 1 mL aliquots of suspension into cryovials.
- Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
- Transfer frozen cells to liquid nitrogen, we recommend vapor phase storage at -200 °C to -125 °C.

Related Products

| Product | Catalog Number |
|----------------------|----------------|
| ESF 921 | 96-001 |
| ESF AF | 99-300 |
| Adapted 293-ES Cells | 94-007 |
| Adapted CHO-ES Cells | 94-008 |

Legend of Labeling Symbols

| Symbol | Interpretation |
|------------------------------------------------------------------------------------|--------------------------|
|  | Catalog Number |
|  | Lot Number |
|  | <i>Research Use Only</i> |
|  | 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

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