Expression Systems
An Advancion Company

CHO-ES Chinese Hamster Ovary Cells
INSTRUCTIONS FOR USE

Product Description

CHO-ES cells are derived from the CHO cell line. CHO-ES cells are adapted for suspension culture in ESF SFM and are available as a frozen vial or suspension culture.
For Research Use Only. Not for use in diagnostic procedures.

Product	Catalog Number	Amount	Storage
CHO-ES cells adapted in ESF SFM, frozen vial	94-008F	20 million cells per vial	Thaw immediately or LN_2
CHO-ES cells adapted in ESF SFM, suspension culture	94-008S	20 million cells in 15 mL media	Culture immediately

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): CHO-ES

Culture Type: Suspension or adherent

Recommended Culture Vessels: Shake flasks (vented is recommended) or T-flask

Temperature Range: 37°C

Incubator Atmosphere: Humidified, 5% CO_2 atmosphere. Ensure proper gas exchange and minimize exposure of cultures to light.

Receiving Frozen Cells

CHO-ES cells are frozen in ESF SFM with 10% DMSO. There are 20 x 10^6 cells per vial.

1. Prepare for thawing cells by placing 20 mL of pre-warmed ESF SFM into a 125 mL vented 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. Transfer contents of vial to culture flaks using a 2 mL pipette. Do not pour.
4. Incubate overnight at 37ºC in a humidified, 5% CO2 shaking incubator. Determine count and viability and bring volume up to 40 mL total using ESF SFM.

Receiving Suspension Cultures

CHO-ES cells are packaged in a 15 mL conical filled to the top with ESF SFM. There are 20 x 10^6 cells per conical.

1. Take extreme care when removing the lid of the conical.
2. Transfer the contents of the conical to a 125 mL vented Erlenmeyer shake flask using a 10 mL pipette. Do not pour. Bring the volume up to 40 mL with pre-warmed ESF SFM.

Suspension Cell Culture

CHO-ES			
Max Density	>4x10^6/mL	Split Density	3-4x10^6/mL
Seed Density	0.5x10^6/mL	Split Frequency	3x/week

Passage the cells three days a week on a Mon/Wed/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 0.5 x 10^6 cell/mL. Use 30-40 mL for a 125 mL Erlenmeyer shake flask.
3. Incubate at 37°C in a humidified, 5% CO_2 atmosphere incubator. Rotate shake flask cultures on an orbital shaker platform at 140-150 rpm. Use vented 125 mL Erlenmeyer flasks to allow for gas exchange (recommended) and loosen caps.
4. Passage when viable cell density reaches 3-4 x10^6 cells/mL. Cultures will grow to densities in excess of 4 x 10^6 but repeated passage at high densities is not recommended.
5. 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.

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 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.
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 SFM (5 mL per 25 cm^2).
5. Incubate at 37°C in a humidified, 5% CO_2 atmosphere incubator. Loosen caps or use flasks with vented caps (recommended).

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Transfection Protocol

1. 24 hours before transfection, seed CHO cells to 1 x 10^6 cells/mL. Incubate at 32°C until transfection. Transfection is most successful in culture with >95% viability.
2. On the day of transfection, bring transfection reagents to room temperature.
3. Prepare transfection solution as follows:
   - Tube 1: 10% final culture volume of SFM media with 1.2µg/mL DNA.
   - Tube 2: 10% final culture of SFM media with PEI at a ratio of 2.5 PEI to 1 DNA.
4. Incubate both tubes at room temperature for 5 minutes.
5. Add contents of PEI tube to DNA tube and mix gently by tapping tube.
6. Incubate 20 minutes at room temperature.
7. Add prepared transfection solution to the prepared cell culture. Incubate at 32°C, shaking at 140-150rpm.
8. Harvest at 72-120 hours post transfection.

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.

Related Products

Product	Catalog Number
ESF SFM	98-001
ESF 921	96-001
Adapted 293 Cells	94-007

Cryopreservation

1. Freezing medium is sterile filtered 90% ESF SFM 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 10-20 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.

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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