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 spinner bottle
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. Prepare for thawing cells by placing 20 ml pre-warmed ESF SFM into a 125 ml vented Erlenmeyer shake flask. Thaw frozen cells rapidly by shaking in a 37ºC water bath. Thaw vial until a small amount of ice remains. Do not leave vial unattended. Transfer contents of vial to culture flaks using a 1 ml pipette. Do not pour. 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. Take extreme care when removing the lid of the conical. 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

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 between 0.2-0.3 x 10^6 cell/mL. Use 30-40 mL for a 125 mL Erlenmeyer shake flask, 50-75 mL for 100 mL spinner bottle.
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) or loosen caps. 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 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. 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 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).

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.

Related Products

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

Legend of Labeling Symbols

Symbol	Interpretation
REF	Catalog Number
LOT	Lot Number
RUO	*Research Use Only*
	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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