


ExpiCHO-S™ Cells and ExpiCHO™ Expression Medium

Catalog Numbers A29127, A29132, A29100-01, A29100-02, A29100-03, and A29100-04

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 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Description

The ExpiCHO-S™ cell line is a clonal derivative of the CHO-S™ cell line that has been selected for high protein expression. ExpiCHO-S™ cells are adapted to high-density, serum-free suspension culture in ExpiCHO™ Expression Medium. The cells can be thawed directly into ExpiCHO™ Expression Medium, and transfection and expression experiments may be performed in ExpiCHO™ Expression Medium without the need for media change.

ExpiCHO™ Expression Medium is a chemically-defined serum-free medium developed specifically for the high-density culture and transfection of ExpiCHO-S™ cells in **suspension**. The medium is designed for scalable transient transfection and protein expression, and does not interfere with nor reduce the activity of ExpiFectamine™ CHO™ Reagent. As a complete, ready-to-use medium formulated with GlutaMAX™-I reagent, ExpiCHO™ Expression Medium requires no additional supplementation.

Product	Catalog no.	Amount	Storage
ExpiCHO-S™ Cells (1 × 10 ⁷ cells/mL)	A29127	1 mL	Liquid nitrogen vapor phase
	A29132	6 × 1 mL	
ExpiCHO™ Expression Medium	A29100-01	1 L	2°C to 8°C, Protect from Light
	A29100-02	6 × 1 L	
	A29100-03	10 L	
	A29100-04	20 L	

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at -80°C.**
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.
- Prior to starting experiments, ensure you have established cells and have frozen stocks on hand. Upon receipt, grow and freeze multiple vials of cells to ensure that you have an adequate supply of early-passage cells.

Culture conditions

Media: ExpiCHO™ Expression Medium

Culture type: Suspension

Temperature range: 36.5°C ± 5°C

Shaker speed: For shakers with a 19-mm throw, set the shake speed to 125 ± 5rpm. For shakers with a 25-mm throw, set the shake speed to 120 ± 5rpm. For shakers with a 50-mm throw, set the shake speed to 95 ± 5rpm.

Incubator atmosphere: Humidified atmosphere of 8% CO₂. Ensure that proper gas exchange is achieved in culture vessels.

Prepare medium

ExpiCHO™ Expression Medium is formulated with GlutaMAX™-I reagent. For suspension growth and transfection applications, use the ExpiCHO™ Expression Medium without any supplementation.

Guidelines for ExpiCHO-S™ cell culture

- ExpiCHO-S™ is a robust cell line adapted to high density growth conditions with a doubling time of approximately 17 hours.

- The cells have a broad log-phase growth window spanning approximately 4×10^6 – 15×10^6 cells/mL with a maximum density of $\geq 20 \times 10^6$ cells/mL in shake flask cultures.
- For general maintenance of cells, passage ExpiCHO-S™ cells when they reach a density of approximately 4×10^6 – 6×10^6 viable cells/mL (i.e. early log-phase growth), typically every 3–4 days.
- Cells that are subcultured at densities outside of this early log-phase growth window may show longer doubling times and lower titers over time. If necessary, modify the initial seeding density to attain the target cell density of 4×10^6 – 6×10^6 viable cells/mL at the time of subculturing.
- Use a hemocytometer with the trypan blue exclusion method or an automated cell counter to determine cell viability. Log phase cultures should be >95% viable.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.

Thaw ExpiCHO-S™ cells

1. Remove the vial of cells from liquid nitrogen and swirl in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains. Do **not** submerge the vial in the water.
2. Just before the cells are completely thawed, decontaminate the vial by wiping it with 70% ethanol before opening it in a laminar flow hood.
3. Using a 2-mL or 5-mL pipette, transfer the entire contents of the cryovial **drop-wise** into a 125-mL polycarbonate or PETG, disposable, sterile, vent-cap Erlenmeyer shaker flask containing 30 mL of pre-warmed ExpiCHO™ Expression Medium.

Note: Do not centrifuge the contents of the cryovial. Centrifugation can be detrimental to ExpiCHO-S™ cell recovery. The small amount of DMSO that will remain in the culture during the thawing process should not affect cell viability.
4. Determine viable cell density. Use a hemocytometer and trypan blue exclusion method or automated cell counter. Cell density should be $\sim 0.2 \times 10^6$ – 0.5×10^6 cell/mL.
5. Incubate the cells in a 37°C incubator with humidified atmosphere of 8% CO₂ on an orbital shaker platform.
6. Three days post-thaw, determine viable cell density and percent viability. Cell viability should be $\geq 90\%$ by three days post-thaw.
7. Continue to monitor cell density and viability and subculture the cells once the culture has reached 4×10^6 – 6×10^6 viable cells/mL (typically 3–4 days post-thaw).

Subculture ExpiCHO-S™ cells

Subculture ExpiCHO-S™ cells when they attain a minimum density of 4×10^6 – 6×10^6 viable cells/mL. Cells should exhibit only minimal clumping during routine cell culture maintenance.

1. Using the viable cell density, calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities in Table 1 and the recommended culture volumes in Table 2.

Table 1 Recommended seeding densities for routine cell culture

Sub-culture timing	Seeding density
Cells ready 3 days post-passage	0.2×10^6 – 0.3×10^6 viable cells/mL
Cells ready 4 days post-passage	0.1×10^6 – 0.2×10^6 viable cells/mL

Table 2 Recommended culture volumes for various flask sizes

Flask size	Recommended culture volume
125-mL	30–35 mL
250-mL	60–70 mL
500-mL	120–140 mL
1-L	240–260 mL

2. Transfer the calculated volume of cells to fresh, pre-warmed ExpiCHO™ Expression Medium in a shake flask.
3. Incubate flasks in a 37°C incubator with a humidified atmosphere of 8% CO₂ on an orbital shaker platform until cultures reach a density of 4×10^6 – 6×10^6 viable cells/mL.

Note: If necessary, modify the initial seeding density to attain the target cell density of 4×10^6 – 6×10^6 viable cells/mL at the time of subculturing.
4. Repeat Steps 1 on page 2–3 on page 2 to maintain or expand the cells for transfection.

Cryopreservation











ExpiCHO-S™ cells can be frozen directly in ExpiCHO™ Expression Medium. When freezing ExpiCHO-S™ cells, follow the recommendations below:

- Freeze ExpiCHO-S™ cells at a final density of 1×10^7 viable cells/mL in 1 mL total volume of 90% fresh ExpiCHO™ Expression Medium and 10% DMSO.
- Allow cells to attain a viable cell density of 4×10^6 – 6×10^6 cells/mL and >95% viability before harvest.
- Centrifuge the cells at $300 \times g$ for 5 minutes to pellet, discard the spent medium, and replace it with ice cold ExpiCHO™ Expression Medium with 10% DMSO. Gently resuspend the cell pellet by pipetting.
- Dilute the cells to a final density of 1×10^7 viable cells/mL and aliquot 1 mL per cryovial.
- Freeze the cells in an automated or manual controlled-rate freezing apparatus following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
- Transfer frozen vials to liquid nitrogen for long-term storage.

Related products

Product	Cat. no.
ExpiCHO™ Expression System	A29133
ExpiFectamine™ CHO™ Transfection Kit for 1 L of culture	A29129
ExpiFectamine™ CHO™ Transfection Kit for 10 L of culture	A29130
ExpiFectamine™ CHO™ Transfection Kit for 50 L of culture	A29131
OptiPRO™ SFM™	12309
Antibody Expressing Positive Control Vector	A14662
pcDNA™ 3.4-TOPO™ TA Cloning™ Kit	A14308
Trypan Blue Stain	15250

Explanation of symbols and warnings

				
Caution, consult accompanying documents	Temperature Limitation	Keep away from light	Use By:	Consult instructions for use
				
Batch Code	Catalog number	Manufacturer	Sterilized using aseptic processing techniques	Read Safety Data Sheet

Limited product warranty

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Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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