ExpiCHO-S™ Cells (cGMP Banked)

Catalog Number A37785

Pub. No. MAN0017984 **Rev.** A.0

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**.

Product description

The Gibco™ 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. Transfection and expression experiments may be performed in ExpiCHO™ Expression Medium without the need for media change.

Contents and storage

Contents	Amount	Storage
ExpiCHO-S™ Cells (cGMP Banked)	1 vial (~1 × 10^7 cells)	Liquid nitrogen vapor-phase[1]

[1] Do not store the cells at –80°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
ExpiCHO™ Expression Medium	A2910001
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	4115-0125
Orbital shaker in temperature and CO_2 controlled incubator	MLS
Trypan Blue	15250

ExpiCHO-S™ Cells characteristics

Growth properties: Suspension

Temperature range: 37°C

Note: Do not grow cells at temperatures above 37°C.

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

Incubator atmosphere: Humidified atmosphere of 8% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Growth characteristics: The cells have a broad log-phase growth window spanning approximately 4 × 10^6–15 × 10^6 cells/mL with a maximum density of ≥20 × 10^6 cells/mL in shake flask cultures.

Doubling time: Approximately 17 hours during log phase growth. Doubling times may vary based on cell health, handling, and passage number.

Viability: Monitor cell growth and viability the first 3–4 days to ensure the cells are not compromised. Cell viability should be ≥90% by 3–4 days post-thaw.

Subculture conditions: Grow cells to 4 × 10^6–6 × 10^6 viable cells/mL; then, split cells to 0.15 × 10^6–0.2 × 10^6 viable cells/mL for 4 days, or 0.2 × 10^6–0.3 × 10^6 viable cells/mL for 3 days. Do not grow above 6 × 10^6 viable cells/mL during maintenance culture. Discard cells after passage number 20.

Note: Cells that are subcultured at densities outside of early log-phase growth window (i.e., 4 × 10^6–6 × 10^6 viable cells/mL) 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.

Procedural guidelines

- Subculture the ExpiCHO-S™ cells a minimum of two times to allow them to recover from thawing before using them in transfection experiments.
- Keep cell densities between 0.15 × 10^6–6 × 10^6 cells/mL during maintenance culture for best performance.
- When thawing or subculturing cells, transfer cells into pre-warmed ExpiCHO™ Expression Medium.
- We recommend maintaining cells in a 125-mL or a 250-mL polycarbonate or PETG, disposable, sterile Erlenmeyer flask containing 30 mL or 60 mL total working volume of cell suspension, respectively.
- **Thaw and recover**: 3–4 days
- **Subculture**: Every 3–4 days

Guidelines to cryopreserve ExpiCHO-S™ Cells

- ExpiCHO-S™ cells can be frozen directly in ExpiCHO™ Expression Medium.
- Freeze ExpiCHO-S™ cells at a final density of 1 × 10^7 viable cells/mL.
- Use a freezing medium composed 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.

Cryopreserve ExpiCHO-S™ Cells

1. Centrifuge the cells at 300 × 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.
2. Dilute the cells to a final density of 1 × 10^7 viable cells/mL and aliquot 1 mL per cryovial.
3. 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.
4. Transfer frozen vials to liquid nitrogen for long-term storage.

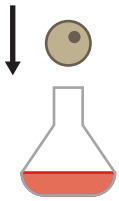
Thaw and passage ExpiCHO-S™ Cells

1 **Day 1: Thaw cells**

Rapidly thaw the cells in a water bath, decontaminate the vial using 70% ethanol, and open the cryovial in a class II biological cabinet.

2 **Day 1: Add cells to medium**

Add cells to 30 mL of pre-warmed ExpiCHO™ Expression Medium in a 125-mL shake flask.



3 **Day 1: Incubate cells**

Incubate cells with the following condition:

Temperature: 37°C

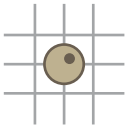
Note: Do not grow cells at temperatures above 37°C.

Humidified atmosphere: 8% CO_2

Orbital shaker platform:

- 125 ±5 rpm (19-mm shaker throw)
- 120 ±5 rpm (25-mm shaker throw)
- 95 ±5 rpm (50-mm shaker throw)

4 **Day 3–4: Count cells and determine viability**



On Day 3 post-thaw, count cells and determine percent cell viability.

Use a hemocytometer and trypan blue exclusion method or automated cell counter. Cell viability should be ≥90%. If viability is not ≥90%, continue to monitor cell viability every day. Split cells when viability is ≥90%.

5 **Day 3–4: Subculture cells**

First passage: When cell density reaches 4×10^6–6×10^6 viable cells/mL at ≥90% viability, split the culture to 0.15×10^6–0.2×10^6 cells/mL (for 4 days passaging schedule) or 0.2×10^6–0.3×10^6 cells/mL (for 3 days passaging schedule) in ExpiCHO™ Expression Medium.

Subsequent passages: Every 3–4 days, cells should reach 4×10^6–6×10^6 cells/mL. Split to 0.15×10^6–0.2×10^6 cells/mL for 4 days passaging schedule, or 0.2×10^6–0.3×10^6 cells/mL for 3 days passaging schedule.

IMPORTANT! Do not grow above 6×10^6 viable cells/mL during maintenance culture.

Note: We recommend maintaining cells in a 125-mL or a 125-mL shake flask containing 30 mL or 60 mL of medium, respectively.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

The information in this guide is subject to change without notice.