

# ExpiCHO-S™ Cells (cGMP Banked)

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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](http://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 <sup>7</sup> cells)	Liquid nitrogen vapor-phase <sup>[1]</sup>

<sup>[1]</sup> Do not store the cells at -80°C

## Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). MLS: Fisher Scientific ([fisherscientific.com](http://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 <sub>2</sub> 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<sub>2</sub>. 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<sup>6</sup>–15 × 10<sup>6</sup> cells/mL with a maximum density of ≥20 × 10<sup>6</sup> 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<sup>6</sup>–6 × 10<sup>6</sup> viable cells/mL; then, split cells to 0.15 × 10<sup>6</sup>–0.2 × 10<sup>6</sup> viable cells/mL for 4 days, or 0.2 × 10<sup>6</sup>–0.3 × 10<sup>6</sup> viable cells/mL for 3 days. Do not grow above 6 × 10<sup>6</sup> 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<sup>6</sup>–6 × 10<sup>6</sup> 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<sup>6</sup>–6 × 10<sup>6</sup> 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<sup>6</sup>–6 × 10<sup>6</sup> 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<sup>7</sup> 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<sup>6</sup>–6 × 10<sup>6</sup> cells/mL and >95% viability before harvest.

## Cryopreserve ExpiCHO-S™ Cells

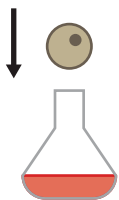
1. Centrifuge the cells at 300 × g for 5 minutes to pellet, discard the spent medium, and replace it with ice cold ExpiCHO™ Expression Medium with 10% DMSO. Gently resuspend the cell pellet by pipetting.
2. Dilute the cells to a final density of 1 × 10<sup>7</sup> viable cells/mL and aliquot 1 mL per 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.



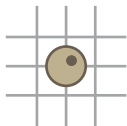
- 2 Day 1: Add cells to medium** Add cells to 30 mL of pre-warmed ExpiCHO™ Expression Medium in a 125-mL shake flask.



- 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<sub>2</sub>  
**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  $4 \times 10^6$ – $6 \times 10^6$  viable cells/mL at ≥90% viability, split the culture to  $0.15 \times 10^6$ – $0.2 \times 10^6$  cells/mL (for 4 days passaging schedule) or  $0.2 \times 10^6$ – $0.3 \times 10^6$  cells/mL (for 3 days passaging schedule) in ExpiCHO™ Expression Medium.



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

**IMPORTANT!** Do not grow above  $6 \times 10^6$  viable cells/mL during maintenance culture.

**Note:** We recommend maintaining cells in a 125-mL or a 250-mL shake flask containing 30 mL or 60 mL of medium, respectively.

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