gibco

ExpiCHO™ Expression System

USER GUIDE

For transfection of ExpiCHO-S™ Cells in a defined, serum-free medium

Catalog Number A29133

Publication Number MAN0014337

Revision D.0

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

ThermoFisher SCIENTIFIC

Manufacturer: Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008

Information in this document is subject to change without notice.

Revision history: Pub No. MAN0014337

Revision	Date	Description
D.0	May 25, 2018	Add products to ordering information, remove LULL 561
C.0	Dec 21, 2016	Update cell culture maintenance volumes. Update troubleshooting. Add additional resources information
B.0	March 2016	Update legal boilerplates; correct volumes in Table 3
A.0	August 2015	New document

Contents

Product information

Product description

ExpiCHO™ Expression System

The ExpiCHO™ Expression System (Cat. no. A29133) is a high-yield transient expression system based on suspension-adapted Chinese Hamster Ovary (CHO) cells. The ExpiCHO™ Expression System Kit provides cells, culture medium, and reagents to transfect 1 liter of cell culture.

Contents and storage

ExpiCHO™ Expression System

The ExpiCHO™ Expression System contains the following components. For a detailed description of each component, see pages 5–6.

Component	Amount	Storage
ExpiCHO-S™ Cells (1 × 10^7 cells/mL)	2 × 1 mL	Liquid nitrogen[1]
ExpiCHO™ Expression Medium	1 liter	2°C to 8°C, Protect from light
ExpiFectamine™ CHO Transfection Kit 1L, contains: ExpiFectamine™ CHO Reagent ExpiCHO™ Feed ExpiFectamine™ CHO Enhancer	1 kit 3 × 1.1 mL 3 × 110 mL 6 mL	2°C to 8°C 2°C to 8°C, Protect from light 2°C to 8°C
OptiPRO™ SFM Complexation Medium	100 mL	2°C to 8°C, Protect from light
Antibody Expressing Positive Control Vector (at 1 mg/mL in TE buffer, pH 8)[2]	150 µg	–20°C

[1] Store the frozen cells in liquid nitrogen until ready to use. Do not store the cells at –80°C.

[2] TE buffer, pH 8.0: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

Components of the ExpiCHO™ Expression System

ExpiCHO™ Expression System

The ExpiCHO™ Expression System is designed to allow high-density transfection of suspension ExpiCHO-S™ cells in a defined, serum-free medium. The system includes ExpiCHO-S™ cells that have been adapted to serum-free, high-density suspension culture in ExpiCHO™ Expression Medium. Transfection and expression experiments may be performed directly in ExpiCHO™ Expression Medium without the need for media change. The ExpiCHO™ Expression System Kit provides cells, culture medium, and reagents to transfect 1 liter of cell culture, as described below. Except for the ExpiCHO-S™ cells, all of the components of the ExpiCHO™ Expression System are animal origin-free.

ExpiCHO-S™ Cells

The ExpiCHO-S™ cell line is a clonal derivative of the CHO-S cell line. The ExpiCHO-S™ cells are adapted to high-density suspension culture in ExpiCHO™ Expression Medium. Frozen cells are supplied in, and may be thawed directly into, ExpiCHO™ Expression Medium.

The ExpiCHO-S™ cell line exhibits the following characteristics:

- Derived from the same parental lineage as the CHO-S cell line
- Rapid recovery post-thaw
- Adapted to high density, serum-free, suspension growth in ExpiCHO™ Expression Medium
- Doubling time of approximately 17 hours
- Reaches maximum cell densities of approximately 20×10^6 cells/mL in shake flask culture
- High protein expression
- Stable transient expression over passages

The ExpiCHO-S™ cells are also available separately; see page 20 for ordering information.

ExpiCHO™ Expression Medium

ExpiCHO™ Expression Medium is a chemically-defined medium developed specifically for the high-density culture and transfection of ExpiCHO-S™ cells in suspension. ExpiCHO™ Expression Medium requires no additional supplementation.

ExpiCHO™ Expression Medium exhibits the following features:

- Formulated as an optimized, chemically-defined, serum-free, protein-free, animal origin-free medium to support the high-density culture and transfection of ExpiCHO-S™ cells in suspension.
- Supplemented with GlutaMAX™-I.
- Does not interfere with nor reduce the activity of ExpiFectamine™ CHO Reagent.
- Designed for scalable transient transfection and protein expression.

The ExpiCHO™ Expression Medium is also available separately; see page 20 for ordering information.

ExpiFectamine™ CHO Reagent

ExpiFectamine™ CHO Reagent is optimized for the transfection of nucleic acids into high density ExpiCHO-S™ cultures.

The ExpiFectamine™ CHO Reagent exhibits the following features:

- Enables high-efficiency transfection of ExpiCHO-S™ cultures maintained in ExpiCHO™ Expression Medium.
- ExpiFectamine™ CHO/plasmid DNA complexes can be added directly to cells in ExpiCHO™ Expression Medium; it is not necessary to remove complexes nor change or add medium following transfection.

  Note: Use of transfection reagents other than the ExpiFectamine™ CHO Reagent to transfect high density ExpiCHO-S™ cultures can lead to substantially reduced performance.

ExpiCHO™ Feed

ExpiCHO™ Feed is an optimized, chemically-defined, serum-free, protein-free, animal origin-free formulation designed to work in conjunction with ExpiCHO™ Expression Medium to support long-term, high-density transient transfections.

ExpiFectamine™ CHO Enhancer

ExpiFectamine™ CHO Enhancer is a proprietary, animal origin-free formulation developed to be used in conjunction with ExpiFectamine™ CHO Reagent and ExpiCHO™ Feed to enhance protein production, resulting in maximal protein yields.

Note: The ExpiFectamine™ CHO Reagent, ExpiCHO™ Feed, and ExpiFectamine™ CHO Enhancer are components of the ExpiFectamine™ CHO Transfection Kits.

The ExpiFectamine™ CHO Transfection Kits are also available separately; see page 20 for ordering information.

OptiPRO™ SFM Complexation Medium

OptiPRO™ SFM Complexation Medium is a serum-free, animal origin-free medium used to complex plasmid DNA with ExpiFectamine™ CHO Reagent, providing high protein expression through efficient transfection.

OptiPRO™ SFM is also available separately; see page 20 for ordering information.

Antibody Expressing Positive Control Vector

Antibody Expressing Positive Control Vector is provided as a positive control for transfection and expression in ExpiCHO-S™ cells. The rabbit IgG that is produced in ExpiCHO-S™ cells after transfection with the control vector is secreted into the ExpiCHO™ Expression Medium, with the optimal yields obtained between 8–10 days for the Standard protocol, 10–12 days for the High Titer protocol, and 12–14 days for the Max Titer protocol. For more information on using the Antibody Expressing Positive Control Vector, see page 18.

Antibody Expressing Positive Control Vector is also available separately; see page 20 for ordering information.

Methods

Guidelines for ExpiCHO-S™ cell culture

General cell handling

Follow the general guidelines below to grow and maintain ExpiCHO-S™ cells.

- **IMPORTANT!** All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood.
- **IMPORTANT!** Store the frozen cells in liquid nitrogen until ready to use. Do not store the cells at –80°C.
- Avoid short-term, extreme temperature changes: when storing cells in liquid nitrogen following receipt on dry ice, allow the cells to remain in liquid nitrogen for 3–4 days prior to thaw.
- For all cell manipulations, mix the cells by gentle swirling; avoid vigorous shaking/pipetting.
- Before starting experiments, be sure to have cells established and also have some frozen stocks on hand. Upon receipt, grow and freeze multiple vials of the ExpiCHO-S™ cell line to ensure that you have an adequate supply of early-passage cells.

  Note: To save time and labor, ExpiCHO-S™ cells are also available in convenient 6-vial "Cell Bank" packs to eliminate the need to prepare frozen stocks and verify your own cell bank. See page 20 for ordering information.
- Allow freshly thawed cells to recover in culture for two or more passages post-thaw before transfecting.
- 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 from approximately 4×10^6–15×10^6 cells/mL with a maximum density of approximately 20×10^6 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.

  Note: Cells that are subcultured at densities outside of this early log-phase growth window may show longer doubling times and lower titers over time. 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.

Media

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

IMPORTANT! ExpiCHO™ Expression Medium is sensitive to light. For optimal results, use and store media protected from light.

Thaw and establish ExpiCHO-S™ cells

Introduction

Follow the protocol below to thaw the ExpiCHO-S™ cells to initiate cell culture. The ExpiCHO-S™ cells are supplied in a vial containing 1 mL of cells at 1×10^7 viable cells/mL in 90% ExpiCHO™ Expression Medium and 10% DMSO. Thaw the cells directly into ExpiCHO™ Expression Medium, pre-warmed to 37°C.

Required materials

- ExpiCHO-S™ cells
- ExpiCHO™ Expression Medium, pre-warmed to 37°C
- 125-mL polycarbonate, disposable, sterile, vented Erlenmeyer shake flask
- Reagents and equipment to determine viable cell density and percent viability (e.g., hemocytometer or an automated cell counter, trypan blue)
- Orbital shaker in a 37°C incubator with ≥80% relative humidity and 8% CO_2

IMPORTANT! Store the frozen cells in liquid nitrogen until ready to use. Do not store the cells at –80°C.

Avoid short-term, extreme temperature changes: when storing cells in liquid nitrogen following receipt on dry ice, allow the cells to remain in liquid nitrogen for 3–4 days prior to thaw.

For all cell manipulations, mix cells by gentle swirling and avoid vigorous shaking/pipetting.

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 into a 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask containing 30 mL of pre-warmed ExpiCHO™ Expression Medium.
4. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform.

   Note: Set the shake speed to 125 ± 5 rpm for shakers with a 19mm shaking diameter,
   120 ± 5 rpm for shakers with a 25mm shaking diameter and 95 ± 5 for shakers with a 50mm shaking diameter.
5. Three days post-thaw, determine viable cell density and percent viability. Cell viability should be ≥90% by three days post-thaw.
6. 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) using the procedure on page 9.

Subculture ExpiCHO-S™ cells

Introduction

ExpiCHO-S™ cells are capable of achieving high cell densities; therefore, we recommend that the cells attain a minimum density of 4×10^6–6×10^6 viable cells/mL at the time of subculturing. Cells may exhibit some clumping during routine cell culture maintenance when they reach higher densities. Clumping will not reduce performance of the cells for transfections.

Required materials

- ExpiCHO-S™ cell cultures at 4×10^6–6×10^6 viable cells/mL
- ExpiCHO™ Expression Medium
- Polycarbonate, disposable, sterile, vented Erlenmeyer shake flask (see Table 2)
- Reagents and equipment to determine viable cell density and percent viability (e.g., hemocytometer or an automated cell counter, trypan blue)
- Orbital shaker in a 37°C incubator with ≥80% relative humidity and 8% CO_2

Passage ExpiCHO-S™ cells

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 maintenance

Sub-culture timing	Recommended seeding density
For cells ready 3 days post-subculture	0.2×10^6–0.3×10^6 viable cells/mL
For cells ready 4 days post-subculture	0.15×10^6–0.2×10^6 viable cells/mL

Table 2. Recommended volumes for routine cell culture maintenance

Flask size	125 mL	250 mL	500 mL	1 L	2 L	3 L
Culture volume (mL)	30–35	60–70	120–140	240–280	500–560	600–1000
Shake speed[1]	125 ± 5 rpm (19-mm shaking diameter) 120 ± 5 rpm (25-mm shaking diameter) 95 ± 5 rpm (50-mm shaking diameter)					90± 5 rpm 85± 5 rpm 80± 5 rpm
Flask type	Vented, non-baffled					

[1] Use higher range of recommended shake speeds for larger culture volumes shown above.

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 ≥80% relative humidity and 8% CO_2 on an orbital shaker platform until cultures reach a density of 4×10^6–6×10^6 viable cells/mL.

   Note: Cells that are subcultured at densities outside of this early log-phase growth window may show longer doubling times and lower titers over time. 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.

   Note: For routine cell growth in 125 mL – 2 L shake flasks, set the shake speed to 125 ± 5 rpm for shakers with a 19mm shaking diameter, 120 ± 5 rpm for shakers with a 25mm shaking diameter and 95 ± 5 for shakers with a 50mm shaking diameter.

   Note: For 3 L shake flasks, set the shake speed to 90 ± 5 rpm for shakers with a 19mm shaking diameter, 85 ± 5 rpm for shakers with a 25mm shaking diameter and 80 ± 5 for shakers with a 50mm shaking diameter.

4. Repeat Steps 1–3 to maintain or expand the cells for transfection. See page 10 for recommendations on freezing the cells.

Cryopreservation

ExpiCHO-S™ 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 × *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.

Transfect ExpiCHO-S™ cells

Introduction

For optimal transfection of high-density suspension ExpiCHO-S™ cultures, use the ExpiFectamine™ CHO Reagent included in the transfection kit. Unlike some other serum-free media formulations, ExpiCHO™ Expression Medium does not inhibit transfection. ExpiCHO™ Expression Medium is specifically formulated to enable transfection **without** the need to change or add media.

Required materials

- ExpiCHO-S™ cells cultured in ExpiCHO™ Expression Medium
- Plasmid DNA preparation, sterile, free from phenol and sodium chloride, and containing mostly supercoiled DNA

  Note: We recommend isolating plasmid DNA using the PureLink™ HiPure Plasmid Kit (see page 20 for ordering information). To ensure sterility, you can filter your DNA preparation through a 0.22-µm filter before use.
- Antibody Expressing Positive Control Vector (100 µL of the 1 mg/mL solution provided in the kit)
- ExpiFectamine™ CHO Reagent (Use cold at 4°C; do **not** pre-warm)
- OptiPRO™ SFM complexation medium (Use cold at 4°C; do **not** pre-warm)
- ExpiCHO™ Expression Medium, pre-warmed to 37°C

  Note: Do **not** add antibiotics to media during transfection because it may decrease transfection efficiency.
- Polycarbonate, disposable, sterile Erlenmeyer flasks
- Orbital shaker in a 37°C incubator with ≥80% relative humidity and 8% CO_2
- *Optional*: Orbital shaker in a 32°C incubator with a humidified atmosphere of 5% CO_2 for High Titer and Max Titer protocols (see Step 9, page 15)
- Reagents and equipment to determine viable cell density and percent viability

Guidelines for transfection

- Allow freshly thawed cells to recover in culture for two or more passages post-thaw before transfecting.
- During all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health is critical to maximal performance.
- Use of transfection reagents other than the ExpiFectamine™ CHO Reagent to transfect high density ExpiCHO-S™ cultures can lead to substantially reduced performance.
- Gently invert the ExpiFectamine™ CHO Reagent 4– 5 times before use to ensure thorough mixing.
- Dilute the ExpiFectamine™ CHO Reagent with cold OptiPRO™ Medium just prior to addition to the diluted DNA. Holding diluted ExpiFectamine™ CHO Reagent for longer than 5 minutes before addition to diluted plasmid DNA can lead to reduced protein titers. See Troubleshooting section for additional information.
- Complexation of plasmid DNA and ExpiFectamine™ CHO Reagent takes place at room temperature using cold reagents (4°C).
- Once combined, you can add the ExpiFectamine™ CHO/DNA complexes t the cells immediately or wait for up to 5 minutes without any loss of performance. Longer hold times (up to 10 minutes) may lead to slight losse in performance. Hold times over 10 minutes are not recommended.

- For maximal flexibility, the ExpiCHO™ Expression System offers three different expression protocols (for more information, see Table 3, page 13):
- **Standard Protocol:** Addition of ExpiFectamine™ CHO Enhancer and single feed on Day 1 post-transfection; maintain cells at 37°C throughout the expression run.
- **High Titer Protocol:** Addition of ExpiFectamine™ CHO Enhancer and single feed on Day 1 post-transfection; shift cells to 32°C on Day 1 post-transfection.
- **Max Titer Protocol:** Addition of ExpiFectamine™ CHO Enhancer and first feed on Day 1 followed by a second feed on Day 5 post-transfection; shift cells to 32°C on Day 1 post-transfection.

Note: For many proteins, titers obtained using the High Titer and Max Titer Protocols are 2–3X greater than those obtained with the Standard Protocol; however, some proteins express similarly, or better, using the Standard Protocol depending on the nature of the protein. The choice of protocol should be determined based on the requirements for the specific protein to be expressed and experience with that particular protein.

Scale up transfections

The ExpiCHO™ Expression System is directly scalable from 125-mL to 2-L flask sizes. For large flasks sizes (i.e., 3-L flasks), the shaking speed of the cultures must be lowered (see Table 3).

Table 3. Recommended volumes for transfection at various scales

Flask size	125 mL	250 mL	500 mL	1 L	2 L	3 L
Number of cells required	1.5×10^8	3.0×10^8	6.0×10^8	1.2×10^9	2.4×10^9	4.5×10^9
Culture volume to transfect	25 mL	50 mL	100 mL	200 mL	400 mL	750 mL
Shake speed[1]	125 ± 5 rpm (19-mm shaking diameter) 120 ± 5 rpm (25-mm shaking diameter) 95 ± 5 rpm (50-mm shaking diameter)					**75**± 5 **rpm** **80**± 5 **rpm** **80**± 5 **rpm**
Flask type	Vented, non-baffled					
Amount of plasmid DNA	0.5–1.0 µg total plasmid DNA per mL of culture volume to transfect					
Volume of plasmid DNA[2]	20 µL	40 µL	80 µL	160 µL	320 µL	600 µL
OptiPRO™ SFM[3]	1 mL	2 mL	4 mL	8 mL	16 mL	30 mL
ExpiFectamine™ CHO Reagent	80 µL	160 µL	320 µL	640 µL	1280 µL	2400 µL
OptiPRO™ SFM[4]	920 µL	1.84 mL	3.7 mL	7.4 mL	14.8 mL	28 mL
ExpiCHO™ Enhancer	150 µL	300 µL	600 µL	1200 µL	2400 µL	4500 µL
ExpiCHO™ Feed (Standard)[5]	6 mL	12 mL	24 mL	48 mL	96 mL	180 mL
ExpiCHO™ Feed (High Titer)[5]	6 mL	12 mL	24 mL	48 mL	96 mL	180 mL
ExpiCHO™ Feed (Max Titer)[6]	4 mL on Days 1 and 5	8 mL on Days 1 and 5	16 mL on Days 1 and 5	32 mL on Days 1 and 5	64 mL on Days 1 and 5	120 mL on Days 1 and 5
Final culture volume	~35 mL	~70 mL	~140 mL	~280 mL	~560 mL	~1 L

[1] Recommended shake speed ranges; optimal shake speed should be determined empirically based on the specific laboratory equipment used. Also see Troubleshooting on page 16.

[2] Assuming a plasmid DNA stock concentration of 1mg/mL and a final concentration of 0.8 µg plasmid DNA per mL of culture volume to transfect

[3] Volume of OptiPRO™ SFM used to dilute plasmid DNA

[4] Volume of OptiPRO™ SFM used to dilute ExpiFectamine™ CHO Reagent

[5] Feed represents 24% of the initial culture volume

[6] Each feed represents 16% of the initial culture volume

Transfect ExpiCHO-S™ cells

During all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health is critical to maximal performance.

Refer to Table 3, page 13, for suggested volumes for transfection at various scales.

1. Subculture and expand ExpiCHO-S™ cells until the cells reach a density of approximately 4×10^6–6×10^6 viable cells/mL.

Day –1: Split cells

2. On the day prior to transfection (Day –1), split the ExpiCHO-S™ culture from Step 1 to a final density of 3×10^6–4×10^6 viable cells/mL and allow the cells to grow overnight.

Day 0: Transfect cells

3. On the next day (Day 0), determine viable cell density and percent viability. The cells should have reached a density of approximately 7×10^6–10×10^6 viable cells/mL. Viability should be 95–99% to proceed with transfection.
4. Dilute the cells from Step 2 to a final density of 6×10^6 viable cells/mL with fresh ExpiCHO™ Expression Medium, pre-warmed to 37°C. Swirl the flasks gently to mix the cells.

   Note: Discard the remaining cells; do not re-use high density cells for routine subculturing.
5. Prepare ExpiFectamine™ CHO/plasmid DNA complexes using cold reagents (4°C), as described below. It is not necessary to keep reagents on ice during complexation. Simply remove reagents from refrigeration and commence with DNA complexation.

   Note: Total plasmid DNA in the range of 0.5–1.0 µg per mL of culture volume to be transfected is appropriate for most proteins.

   a) Gently invert the ExpiFectamine™ CHO Reagent bottle 4–5 times to mix.
   b) Dilute plasmid DNA with cold OptiPRO™ medium. Mix by swirling the tube and/or by inversion.
   c) Dilute ExpiFectamine™ CHO Reagent with OptiPRO™ medium. Mix by swirling the tube and/or by inversion or gentel pipetting 2–3 times.

      Note: Dilute the ExpiFectamine™ CHO reagent with cold OptiPRO™medium just prior to addition to the diluted DNA. Holding diluted ExpiFectamine™ CHO reagent for longer than 5 minutes before addition to diluted plasmid DNA can lead to reduced protein titers. See Troubleshooting section for additional information.
   d) Add the diluted ExpiFectamine™ CHO Reagent to diluted DNA. Mix by swirling the tube or by inversion.
6. Incubate ExpiFectamine™ CHO/plasmid DNA complexes (from Step 5d) at room temperature for 1–5 minutes, and then slowly transfer the solution to the shaker flask from Step 4, swirling the flask gently during addition.
7. Incubate the cells in a 37° C incubator with a humidified atmosphere of 8% CO_2 in air on an orbital shaker (refer to Table 3, page 13, for suggested shake speeds).

Day 1: Add ExpiFectamine™ CHO Enhancer and ExpiCHO™ Feed

8. On the day after transfection (Day 1, 18–22 hours post-transfection), perform the following additions depending on the protocol chosen (see Table 3, page 13, for the volumes to add):

   Note: It is not necessary to pre-warm the ExpiFectamine™ CHO Enhancer or the ExpiCHO™ Feed prior to addition to flasks.

   Note: ExpiFectamine™ CHO Enhancer and ExpiCHO™ Feed may be pre-mixed together immediately prior to adding to flasks.

   - **Standard Protocol:** Add ExpiFectamine™ CHO Enhancer and ExpiCHO™ Feed to the flask (according to Table 3, page 13), gently swirling the flask during addition. Return the flask to the 37°C incubator with a humidified atmosphere of 8% CO_2 with shaking.
   - **High Titer Protocol:** Add ExpiFectamine™ CHO Enhancer and ExpiCHO™ Feed to the flask (according to Table 3, page 13), gently swirling the flask during addition. Transfer the flask to a 32°C incubator with a humidified atmosphere of 5% CO_2 in air with shaking.
   - **Max Titer Protocol:** Add ExpiFectamine™ CHO Enhancer and ExpiCHO™ Feed to the flask (according to Table 3, page 13), gently swirling the flask during addition. Transfer flask to a 32°C incubator with a humidified atmosphere of 5% CO_2 in air with shaking.

Day 5:

9. **For Max Titer Protocol Only:** On Day 5 post-transfection, add the second volume of ExpiCHO™ Feed to the flask (according to Table 3, page 13) and immediately return the flask to 32°C incubator with shaking.
10. Optimal time to harvest protein will depend on the specific properties of the protein being expressed and the protocol chosen. Typical harvest times to reach maximum titers for the various protocols are as follows:
    - **Standard Protocol:** 8–10 days post-transfection
    - **High Titer Protocol:** 10–12 days post-transfection
    - **Max Titer Protocol:** 12–14 days post-transfection

Optimize protein expression

- Expression levels will vary depending on the specific recombinant protein expressed and the vector used; however, the ExpiCHO™ Expression System will exhibit consistent expression level for any particular protein from one transfection to the next.
- When expressing a protein for the first time, you may want to perform a time course (e.g., harvest cells or media at several time points post-transfection) to optimize the length of the expression run.
- The ExpiCHO™ Expression Medium is designed to support transiently transfected cultures for up to 14 days in conjunction with ExpiFectamine™ CHO Enhancer and ExpiCHO™ Feed in the Max Titer protocol, however, it will not always be necessary, or desirable, to take expression runs out to 14 days for a given protein.

Troubleshooting

EQUIPMENT

- For optimal performance, it is critical that the shaking diameter, shaking speed, flask size/type and volume of culture to be transfected match the recommendations in this protocol for both routine sub culture and protein expression runs.
- Humidified incubators (≥80% relative humidity) are recommended to reduce evaporation during expression runs. When using multi-well plates, high humidity settings should be used if available.
- Ensure that equipment is calibrated for temperature. In some instances, the total heat from the incubator and the shaker can cause cell culture temperatures to exceed the recommended ranges and lead to decreased cell growth, clumping or cell death. In such instances, reduce the temperature setting of the incubator to compensate for heat generated by the shaker.
- Ensure that equipment is calibrated for CO_2. Levels of CO_2 should not exceed 8%.

CELLS

- Cells should recover rapidly post-thaw and exhibit growth profiles within the guidelines of the protocol during routine cell culture maintenance for 3 – 4 days (see page 9).
- ExpiCHO-S™ is a high density cell line: sub culture cells when density has reached log phase growth at 4 – 6 x10^6 viable cells/mL. Sub culturing cells before they have reached log phase growth can negatively impact cell performance.
- During all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting, especially immediately before transfection. Cell health prior to transfection is critical to maximal performance.
- Minimal cell clumping may be observed as cells reach higher cell densities. In such instances simply allow the clumps to settle and take cells from the supernatant; do not attempt to break up clumps.
- Always keep dedicated cell culture maintenance flasks: do not re-purpose remaining high density cells from a transfection run for routine sub culturing.

If cells significantly overgrow target of 4 – 6 × 10^6 viable cells/mL during routine sub culturing (i.e. >8 × 10^6 viable cells/mL), split cells back down to 0.5 × 10^6 viable cells/mL during next passaging to reduce stress on the cells.

PLASMID DNA COMPLEXATION

- Plasmid DNA complexation is optimal using cold reagents at approximately 4°C.
- Plasmid DNA is highly stable in OptiPro™ complexation medium; dilute plasmid DNA prior to dilution of ExpiFectamine™ CHO transfection reagent.
- Once ExpiFectamine™ CHO transfection reagent is diluted with OptiPro™ medium; mix by swirling the tube and/or inversion or gentle pipetting 2–3 times. Do not vortex.
    - Holding the diluted ExpiFectamine™ CHO reagent for longer than 5 minutes before addition to plasmid DNA can lead to reduced protein titers.
- For optimal performance, add plasmid DNA complexes to cell cultures to be transfected within 1–5 minutes post-complexation by drop-wise addition to

the flasks with swirling.

- As an alternative method of complexation, plasmid DNA may be diluted with the entire volume of OptiPRO™ that is typically used to dilute both the plasmid DNA and the ExpiFectamine™ CHO transfection reagent and held until such time as non-diluted ExpiFectamine™ CHO reagent is added to the plasmid DNA to initiate complexation.
    - This alternative method of complexation is useful when automating the ExpiCHO™ protocol or when performing small scales transfections, see Additional resources, page 18

HARVEST

- For typical proteins, high cell viability (ideally 75% or greater) at the time of protein harvest (i.e., 8–10 days for the Standard protocol, 10–12 days for the High Titer protocol, and 12–14 days for the Max Titer protocol) is the primary indicator of maximal ExpiCHO™ system performance. If cell viability is significantly outside of this range, consider the following:
    - Optimize equipment: ensure shake speed, flask size/type and volume to be transfected are within the recommended guidelines.
        - Smaller shake flasks (i.e., 125-mL shake flasks) may benefit from faster shake speeds (i.e., 140 rpm for a shaker with a 19-mm shaking diameter). Because instruments and shakers differ from laboratory to laboratory, we recommend that you optimize shake speed based on empirical data.
        - For 125-mL shake flasks, lower flask-to-flask variability may be achieved using baffled flasks. If baffled flasks are used, we recommend increasing the culture volume to 30 mL while maintaining the shaking speed at 125 ± 5 rpm (for shakers with a 19-mm shaking diameter).
    - Optimize cell culture: during all cell manipulations, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health prior to transfection is critical to maximal performance. Ensure that cells are within recommended density ranges during routine sub culturing and at the time of transfection.
    - Optimize the complexation reaction: dilute ExpiFectamine™ CHO reagent with cold OptiPRO™ medium just prior to addition to diluted plasmid DNA. Holding the diluted ExpiFectamine™ CHO reagent for longer than 5 minutes before addition to diluted plasmid DNA can lead to reduced protein titers.

For best performance, add ExpiFectamine™ CHO/plasmid DNA complexes to cultures within 1-5 minutes post-complexation.

CLARIFICATION OF CELL CULTURE SUPERNATANT

- Following harvest, centrifuge the supernatant at 4000 – 5000 x g for 30 minutes in a refrigerated centrifuge.
- Filter supernatant through a 0.22-µm filter.
- For optimal purification of antibodies using Protein A capture, refer to Protein A purification, in Additional resources, page 18.

Additional resources

Essential protocol video
thermofisher.com/expichovideo
Essential Protocol Guide (Frequently Asked Questions)
thermofisher.com/expichoguide
Expi Protocol Calculator
thermofisher.com/expicalculator
Protein A purification application note
thermofisher.com/expichopurification
Small down protocols 24 and 96 deep well blocks and mini bioreactor tubes
thermofisher.com/expichoscaledown

Appendix A: Positive control for transfection and expression

Antibody Expressing Positive Control Vector

Antibody Expressing Positive Control Vector is provided as a positive control for transfection and expression in ExpiCHO-S™ cells. The control contains pcDNA3.4 plasmid clones expressing the heavy and light chains of a rabbit IgG. The control is provided as a ready-to-use transfection-grade plasmid at a concentration of 1 mg/mL with a 2:1 light chain:heavy chain ratio and is sufficient to transfect up to 150 mL of ExpiCHO-S™ cells.

Transfection and expression

Transfect 30 mL of suspension ExpiCHO-S™ cells using 24–30 µL of the Antibody Expressing Positive Control Vector (i.e., 0.8–1 µg of positive control per 1 mL of ExpiCHO-S™ culture) following the protocol provided on pages 14–15.

The rabbit IgG that is produced in ExpiCHO-S™ cells after transfection with the control vector is secreted into the ExpiCHO™ Expression Medium, with optimal yields obtained between 8–10 days for the Standard protocol (200–300 mg/L), 10–12 days for the High Titer protocol (300–500 mg/L), and 12–14 days for the Max Titer protocol (>500 mg/L).

Note: The titer values referenced above were determined in crude cell culture supernatants using a Pall Life Sciences FortéBio™ Octet™ instrument equipped with a protein A biosensor.

Appendix B: Ordering information

Additional products

The following reagents supplied in the ExpiCHO™ Expression System and other accessory products suitable for use with the kit are available separately. Ordering information is provided below. For more information, refer to **thermofisher.com** or contact Technical Support (see page 24).

Product	Amount	Catalog No.
ExpiCHO-S™ Cells (1×10^7 cells/vial)	1 vial	A29127
ExpiCHO-S™ Cells, 6 vial "Cell Bank" pack (1×10^7 cells/vial)	6 vials	A29132
ExpiCHO™ Expression Medium	1 L 6 × 1 L 10 L 20 L	A29100-01 A29100-02 A29100-03 A29100-04
ExpiFectamine™ CHO Transfection Kit for 1 L of culture	1 kit	A29129
ExpiFectamine™ CHO Transfection Kit for 10 L of culture	1 kit	A29130
ExpiFectamine™ CHO Transfection Kit for 50 L of culture (5 × 10 L kit)	1 kit	A29131
OptiPRO™ SFM	100 mL 1000 mL	12309-050 12309-019
Antibody Expressing Positive Control Vector (at 1 mg/mL)	150 µg	A14662
pcDNA™ 3.4-TOPO™ TA Cloning Kit	1 kit	A14308
Trypan Blue Stain	100 mL	15250-061

Plasmid purification products

The following plasmid purification products suitable for use with the ExpiCHO™ Expression System are available separately. For more information, refer to **thermofisher.com** or contact Technical Support (see page 24).

Product	Amount	Catalog No.
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-14
PureLink™ HiPure Plasmid Filter Midiprep Kit	25 preps	K2100-04
PureLink™ HiPure Plasmid Maxiprep Kit	10 preps	K2100-06
PureLink™ HiPure Plasmid Filter Maxiprep Kit	10 preps	K2100-16
PureLink™ HiPure Plasmid Megaprep Kit	4 preps	K2100-08

Visualization and quantitation of control antibody

The following products for visualizing and quantitating the control rabbit IgG antibody expressed from the Antibody Expressing Positive Control Vector are available separately. For more information, refer to **thermofisher.com** or contact Technical Support (see page 24).

Product	Amount	Catalog No.
Protein A	25 mg	101006
F(ab')$_2$ fragment of goat anti rabbit (H+L) horseradish peroxidase conjugate	0.5 mg	A10547
Purified Rabbit IgG	10 mg	02-6102
SimplyBlue™ Safestain	1 L	LC6060
NuPAGE™ Novex™ 4–12% Bis-Tris Gel 1.0 mm, 12-well (10 gels/box)	1 box	NP0322BOX
Ni-NTA Purification System	6 purifications	K950-01
Ni-NTA Agarose	10 mL 25 mL 100 mL	R901-01 R901-15 R901-10
Protein A Agarose	5 mL	15918-014

Cell culture shake flasks

The following Nalgene™ shake flask products have been validated with the ExpiCHO Expression System. These products are available separately. Ordering information is provided below. For more information, refer to **thermofisher.com/ExpiCHO** or contact Technical Support (see page 24).

Item	Amount	Source
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile, 125mL, vented	Case of 24	4115-0125
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile, 250mL, vented	Case of 12	4115-0250
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile, 500mL, vented	Case of 12	4115-0500
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile, 1000mL, vented	Case of 6	4115-1000
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile, 2000mL, vented	Case of 4	4115-2000
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Baffled Bottom: Sterile, 2800mL, vented	Case of 4	4116-2800

Appendix C: Safety

General safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc.). To obtain SDSs, see the "Documentation and support" section in this document (page 24).

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and support" section in this document (page 24).
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! **BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:

  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:

  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

Visit www.thermofisher.com/support for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many application and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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**.