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WELCOME TO THE FUTURE OF TRANSIENT EXPRESSION

Smart start guide for the ExpiCHO system

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ML
250
200
150
100
ML
250
APPROXIMATE VOLUME
200
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100

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ExpiCHO Expression System

Maintain protein quality and function from research to bioproduction

Switching from 293 to CHO cells during drug development may cost you precious time and create uncertainty. Now there's a better way. The Gibco™ ExpiCHO™ Expression System provides the highest protein yields possible in a transient system. That means you can always work in CHO cells, starting from discovery.

Included in the system:

- Two vials of frozen Gibco™ ExpiCHO-S™ Cells
- 1 L of Gibco™ ExpiCHO™ Expression Medium
- One Gibco™ ExpiFectamine™ CHO Transfection Kit, sufficient to transfect 1 L of culture
- Gibco™ OptiPRO™ Serum-Free Medium
- One Gibco™ Antibody-Expressing Positive Control Vector

Smart start tips

Get started quickly with the ExpiCHO Expression System, and maximize protein yields with these tips for optimal performance.

- **Shaking:** Refer to the shake grid (p. 7) to ensure that the orbital diameter, shaking speed, and vessel type are optimal for the volume of culture to be transfected.
- **Cell preparation:** Subculture high-density ExpiCHO-S cells when they have reached log-phase growth (e.g., 4–6 x 10^6 viable cells/mL). Subculturing ExpiCHO-S cells before or after they have reached log-phase growth can negatively impact performance.
- **Cell handling:** During all cell manipulations, mix ExpiCHO-S cells by gentle swirling; avoid vigorous mixing or pipetting, especially immediately before transfection. Cell health prior to transfection is critical for maximal performance.
- **Transfection:** Dilute the ExpiFectamine CHO reagent with cold OptiPRO medium just prior to addition to diluted plasmid DNA. Mix the diluted ExpiFectamine CHO reagent and diluted plasmid DNA by gentle pipetting 2–3 times or gentle inversion; add to cultures 1–5 minutes after complexation.
- **Cell viability:** For typical proteins, cell viability should be high at the time of harvest (ideally ≥75%). Consult the FAQs in this guide for troubleshooting tips if cell viability drops during the expression run.

Shake grid

Vessel size, expression volume, shaking speed, and orbital diameter are all critical factors for optimal protein expression.

Flasks

Flask size	125 mL	250 mL	500 mL	1 L	2 L	2.8–3 L
Culture volume to transfect	25 mL	50 mL	100 mL	200 mL	400 mL	600–750 mL
Ratio, medium:flask volume	1:3.6	1:3.6	1:3.6	1:3.6	1:3.6	1:3.0–3.5
Shaking speed	125 ± 5 rpm (19 mm orbital diameter) 120 ± 5 rpm (25 mm orbital diameter) 95 ± 5 rpm (50 mm orbital diameter)					75 ± 5 rpm 80 ± 5 rpm 80 ± 5 rpm
Flask type	Vented, non-baffled					
Final culture volume	~35 mL	~70 mL	~140 mL	~280 mL	~560 mL	~850 mL–1 L

Deep-well blocks and spin tubes

Vessel size	96 deep-well block	24 deep-well block	50 mL spin tube
Culture volume to transfect	800 µL	2.5 mL	15 mL
Shaking speed	900 ± 50 rpm (3 mm orbital diameter)	225 ± 5 rpm (19 mm orbital diameter) 220 ± 5 rpm (25 mm orbital diameter)	240 ± 5 rpm (19 mm orbital diameter) 220 ± 5 rpm (25 mm orbital diameter)
Vessel type	96 deep-well, round bottom	24 deep-well, rectangular bottom	50 mL spin tube
Final culture volume	1 mL	3.5 mL	20 mL

Frequently asked questions (FAQs)

Getting started

I'm new to the ExpiCHO Expression System and would like additional information to help me get started. Are there any online resources to assist me?

Yes. Please visit **thermofisher.com/expistart** to access our web application and view the protocol video, protocol calculator, and valuable technical resources for the ExpiCHO system. The protocol video will show exactly how expression with the ExpiCHO system is performed in our laboratories. The protocol calculator will do calculations for you and provide you with a custom protocol for your expression run. These are excellent resources for getting started with the system.

Cell handling

How should the ExpiCHO-S Cells be handled upon receipt?

Upon receipt of the cells on dry ice, it is best to either thaw the cells immediately or place the vials into liquid nitrogen storage for ~72 hours to allow the cells to acclimate until the time of thawing; do not store the cells at –80°C.

Once thawed and transferred into prewarmed medium in a vented, non-baffled shake flask, cells should be incubated at 37°C with 8% CO_2 on a shaker platform set to 120 ± 5 rpm for a shaker with a 25 mm orbital diameter, or 125 ± 5 rpm for a 19 mm orbital diameter.

Cells should have high viability at the time of thawing and should recover quickly post-thaw, reaching their normal 18-hour doubling time within 1–2 passages.

Cells that are very clumpy or stringy in appearance or do not recover to a normal growth pattern within 1–2 passages shouldn't be used.

What are the normal growth characteristics of ExpiCHO-S Cells?

Within 1–2 passages post-thaw, ExpiCHO-S cells should be growing with a doubling time of approximately 18 hours. There should be minimal cell clumping in the flask, with only small clumps visible when the cells approach higher densities (i.e., ~10 x 10^6 cells/mL).

When cells are cultured at 0.1–0.3 x 10^6 cells/mL, viable cell density should be approximately 4–6 x 10^6 cells/mL within 3 or 4 days. If cells are not growing within these approximate ranges, cell culture conditions will require further optimization.

I've followed all of the recommendations for thawing my ExpiCHO-S Cells, but they are not growing post-thaw. What can I do?

In instances where ExpiCHO-S Cells are thawed and do not start to grow as noted above—attaining only relatively low densities in culture—one common solution is to verify the temperature of the cultures to ensure that the equipment settings are not generating too much heat. CHO cells are very tolerant of lower temperatures but are more sensitive to elevated temperatures, compared to HEK293 cells. Incubators, even when set to 37°C, in conjunction with the heat generated from the shakers can elevate the temperature in the culture flasks above what is optimal for CHO cells.

The optimal temperature of the medium for ExpiCHO-S cultures is ~36.5°C. If overheating is suspected, reduce the temperature of the incubator to reach optimal operating temperatures in the flasks. In such instances, it is best practice to thaw a new vial of cells rather than attempt to recover cells from elevated temperature conditions. Also, non-baffled flasks are recommended for use at all scales for both routine subculturing and protein expression runs.

What are best practices for handling ExpiCHO-S Cells?

ExpiCHO-S cells do not reach log-phase growth until approximately 3 x 10^6 cells/mL; thus, ExpiCHO-S Cells should be allowed to attain a density of 4–6 x 10^6 cells/mL at the time of subculturing to ensure the cells have reached the log phase. As cells approach high densities (e.g., 10–12 x 10^6 cells/mL), some very small cell clumps may be visible; do not try to break up the clumps, and simply let them settle and remove the cell suspension for use.

For all cell manipulations, simply swirl flasks to resuspend the cells. Do not shake or pipet the cells vigorously to mix, as this can lead to decreased performance, especially just prior to transfection, when cells have attained very high densities.

How soon after thawing can I use my cells, and how many passages are they good for?

ExpiCHO-S cells should be passaged at least twice post-thaw and be growing within the ranges specified in the ExpiCHO expression system manual, prior to transfection. Cells will perform consistently for at least 20 passages if maintained in accordance with the guidelines in the manual.

What if my cells overgrow the target density of 4–6 x 10^6 cells/mL ahead of subculturing?

If the cells significantly overgrow the target of 4–6 x 10^6 cells/mL (i.e., growing to 8–10 x 10^6 cells/mL), simply subculture the cells to a higher cell density than normal (i.e., seed new flasks at ~0.5 x 10^6 cells/mL) to allow the cells to recover.

Since ExpiCHO-S cells are a high-density cell line, we do not recommend subculturing the cells if they have not yet reached log-phase growth at 4–6 x 10^6 cells/mL, as this can impair growth over time.

How can I ensure that my cells are growing optimally?

As a simple check, ExpiCHO-S cells can be seeded at 0.3 x 10^6 cells/mL in 30 mL of ExpiCHO medium in a 125 mL non-baffled flask, and the viability and viable cell density checked on days 5, 6, and 7 post-seeding without changes or addition of medium. ExpiCHO-S cells will typically reach maximal density around day 6 post-seeding, in the range of 20 x 10^6 cells/mL, and then will die off on day 7 and beyond.

Determining the final viable cell density will depend on the method used to count cells. Significant variation can be observed with different counting methods.

If cells are exhibiting significantly different growth profiles, culture conditions should be optimized. It is typical to test several different shaking speeds simultaneously to determine which speed provides optimal cell growth, and then start with this speed for your protein expression runs.

Medium

Can I substitute other media for ExpiCHO medium?

No. The ExpiCHO Expression System achieves very high titers by the way the components of the system have been optimized to work together for maximal protein expression.

ExpiCHO medium is a transfection-compatible, high-density growth medium specifically matched to Gibco™ ExpiCHO™ Feed and ExpiFectamine™ CHO Enhancer.

Other media are not compatible with the ExpiCHO system and may inhibit protein expression altogether.

Transfection

What density should the ExpiCHO-S Cells be at, at the time of transfection?

ExpiCHO-S cells should be subcultured at a density of 3–4 x 10^6 cells/mL one day prior to transfection in order to obtain a cell density of approximately 7–10 x 10^6 cells/mL on the day of transfection. These cells should be diluted to a final density of 6 x 10^6 cells/mL with fresh medium and gently swirled to mix prior to transfection.

Discard any remaining cells; do not reuse high-density cells for seeding of maintenance flasks.

How can I ensure the best complexation of the ExpiFectamine CHO reagent and my plasmid DNA?

For best results, perform DNA complexation in the following manner:

1. Dilute the plasmid DNA in cold OptiPRO medium.
2. At the time of use, dilute the ExpiFectamine CHO reagent with cold OptiPRO medium and then immediately add to the diluted plasmid DNA.
3. Mix by gentle pipetting 1–2 times and/or inversion; do not vortex or pipet vigorously.
4. Complexation time should be between 30 seconds and 5 minutes.

Avoid elongated hold times for the diluted ExpiFectamine CHO reagent or the ExpiFectamine CHO reagent/plasmid DNA complexation mixture.

What if I cannot add diluted ExpiFectamine CHO reagent to the diluted plasmid DNA immediately?

If it is not possible to immediately add diluted ExpiFectamine CHO reagent to the diluted plasmid DNA, we recommend diluting your plasmid DNA in the total complexation volume that would be used according to the kit protocol (i.e., the total volume of OptiPRO medium that would normally be used for diluting both the ExpiFectamine CHO reagent and the plasmid DNA) and then adding undiluted ExpiFectamine CHO reagent directly to the diluted plasmid DNA. Mix by gentle pipetting 1–2 times and/or inversion.

This method is useful for automation or small-scale transfections, where it is impossible or undesirable to add the ExpiFectamine CHO reagent to plasmid DNA immediately after dilution.

Can I really use only 0.5–0.8 µg/mL plasmid DNA? Can lower DNA levels be used for hard-to-express proteins?

ExpiFectamine CHO reagent is a highly efficient transfection reagent, enabling use of significantly lower levels of plasmid DNA for expression runs.

Higher levels of DNA can be more stressful to the cells. The volume of ExpiFectamine CHO reagent specified in the kit protocol will account for using plasmid DNA in the range from 0.5 to 0.8 µg/mL; if using less DNA than this, the amount of ExpiFectamine CHO reagent should be reduced proportionately for best results. It is recommended to use 0.6–0.8 µg/mL for most proteins.

For some proteins that are prone to aggregation or otherwise difficult to express, lower DNA levels may be beneficial to expression.

Are there any tips regarding the optimal proportion of light- and heavy-chain–encoding plasmids for optimal antibody expression?

For the majority of antibodies tested, a 1:1 ratio has been found to work well for the ExpiCHO system. The optimal ratio of heavy- to light-chain plasmids depends on the rate at which the two chains are expressed when the plasmids are cotransfected into the same cell, and may be antibody-specific in some instances.

We recommend starting at 1:1 and then modifying as necessary for specific molecules.

We recommend cloning the heavy- and light-chain subunits separately into the Invitrogen™ pcDNA3.4™ TOPO™ vector initially, and then optimizing the ratios of the two plasmids. We have also tested single plasmids incorporating both heavy- and light-chain genes with comparable results, so in the end, the answer will come down to user experience and preference.

Can I use a different transfection reagent?

The ExpiFectamine CHO Transfection Reagent, ExpiFectamine CHO Enhancer, and ExpiCHO Feed are optimized to work together to provide maximal protein expression levels and are provided in a single kit for convenience.

The ExpiFectamine CHO Transfection Reagent provides high-efficiency transfection of high-density cultures, superior to any other transfection reagent.

Expression levels >30-fold higher are obtained using the ExpiCHO kit as directed, compared to substitution of polyethyleneimine (PEI) for the transfection reagent, while using 50% less DNA in both methods.

Vector selection

Which vector is recommended with this system?

We recommend using the pcDNA3.4 TOPO TA vector (Cat. No. A14697) for optimal expression. This vector contains the native, full-length CMV promoter and a WPRE downstream of the cloning site. Other CMV promoter–based vectors generally offer good levels of expression for CHO and HEK293 transient expression systems. Thus, a variety of CMV promoter plasmids may be suitable for expressing your protein of interest.

When should the ExpiCHO Feed and ExpiFectamine CHO Enhancer be added to the cultures?

ExpiCHO Feed and ExpiFectamine CHO Enhancer should be added 18–22 hours posttransfection for best results. These solutions may be added to the flasks without prewarming. ExpiCHO Feed and ExpiFectamine CHO Enhancer may also be premixed ahead of addition to flasks to reduce the number of steps required. If using the standard protocol at 37°C, we recommend adding the feed and enhancer closer to the 18-hour point.

Are there any special instructions for the high-titer and max-titer protocols?

We recommend that a dedicated 32°C incubator be used for shifting the temperature for the high-titer and max-titer protocols: with simply changing the temperature setting of a 37°C incubator to 32°C, it may take a long time for the cooling to complete and may limit the effectiveness of the temperature shift. Cooling the incubator by opening the door may result in contamination.

Protein harvest

On what day should I harvest my supernatant?

The time of harvest is highly dependent upon the nature of your protein. For stable proteins such as antibodies, we generally recommend harvesting on day 7–8 posttransfection (using the standard protocol), on day 9–10 (using the high-titer protocol), or on day 11–12 (using the max-titer protocol). Cell viability should still be high at this time, ideally 70–80% or greater. Intracellular proteins may require earlier harvesting, between days 4 and 7.

For proteins that may be susceptible to degradation, a time course of harvesting should be considered to identify the optimal takedown time. In all instances, maintaining high cell viability at the time of harvest is ideal for both protein quality and purification.

Sometimes the supernatant is difficult to filter during clarification. How can I overcome this?

The supernatant from ExpiCHO cultures may be more difficult to process through standard bottle-top filters, compared to Expi293 cultures.

To remedy this, we recommend centrifuging the supernatant first at ~5,000 x *g* for 30 minutes, followed by filtration using a 0.22 μm filter. Alternative filters (such as depth filters) provide superior filtration for supernatants, especially at larger scales, as these filters are specifically designed to handle supernatants from CHO-derived expression. Refer to the optimized protocol for supernatant clarification and protein A purification for the ExpiCHO Expression System at **thermofisher.com/expistart**.

System scalability

Can an ExpiCHO expression run be scaled down to smaller volumes than those of shake flasks?

Yes. For expression in 24 and 96 deep-well blocks and 50 mL mini bioreactor tubes, please refer to the protocol at **thermofisher.com/expistart**.

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APPLICATION NOTE

ExpiCHO Expression System

ExpiCHO Expression System

Protocols for 24 and 96 deep well blocks and mini bioreactor tubes

The Gibco™ ExpiCHO™ Expression System brings together a high-expressing CHO cell line and an optimized medium and transfection kit that synergistically act to provide titers as much as 160x higher than the Gibco™ FreeStyle™ MAX CHO Expression System and 4x higher than the Gibco™ Expi293™ Expression System.

The ultrahigh yields of the ExpiCHO Expression System (up to 1–3 g/L for some proteins) allow you to scale down your expression run and achieve significant cost savings compared to other transient expression technologies. Here we present protocols to scale down the system to 24 and 96 deep well blocks and mini bioreactor tubes.

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Cell viability

Sometimes in the high-titer or max-titer protocols I see a drop in viability on about day 7–8 posttransfection. How can I fix this?

This drop in viability indicates that some aspect of the cell culture conditions is not optimal during the expression run. If this is observed, the shaking speed of the flask should be verified to be within protocol specifications; the volume and size of the flask should be appropriate, and care should be taken when handling the ExpiCHO-S cells ahead of the expression run to ensure that cells are not stressed by vigorous mixing.

In some instances, flask-to-flask variation has been observed using the high- and max-titer protocols at the 125 mL flask scale. Here, we recommend increasing the shaking speed to 130 rpm for 25 mm shakers and 140 rpm for 19 mm shakers.

Alternatively, we have found that baffled flasks work well for the 125 mL scale to decrease flask-to-flask variation. When using baffled flasks, the volume of cells to be transfected should be increased from 25 mL to 35–40 mL to allow for optimal flow over the baffles, and the volumes of other reagents should be scaled accordingly.

Lower transfection volumes (i.e., 30–35 mL) can also be used with baffled flasks; however, shaking speeds must be reduced slightly to account for the baffles (115 rpm for 19 mm shakers and 110 rpm for 25 mm shakers). We have not found baffled flasks to be necessary or beneficial at other flask sizes.

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Find out more at **thermofisher.com/expicho**

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