

# Seamlessly transition from transient protein expression to bioproduction with the ExpiCHO system

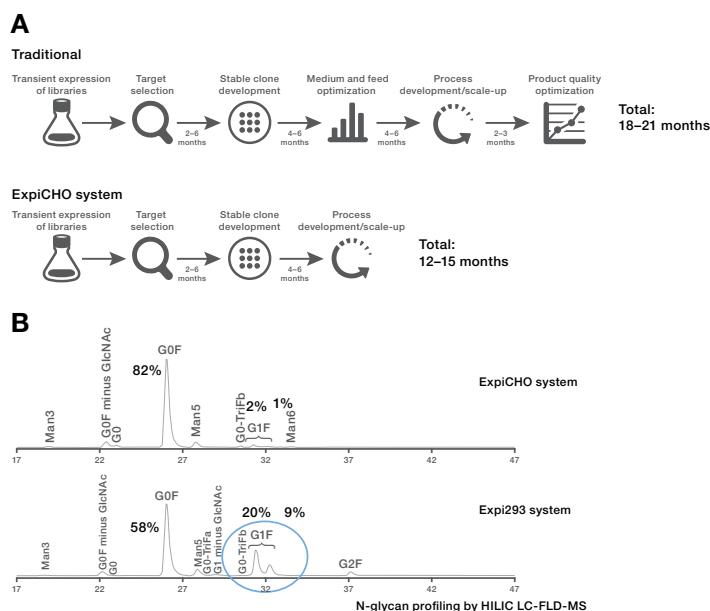
## Introduction

As competition and growth in the biopharmaceutical industry continues, the need to reduce cell line development costs and decrease time-to-market is more crucial than ever. Ideally, the same host cell line would be used from discovery through large-scale manufacturing. The cell line of choice for biopharmaceutical manufacturing is Chinese hamster ovary (CHO) cells. Historically, using these cells in transient systems for rapid production of sufficient quantities of protein for candidate screening has been challenging. Researchers have relied on alternative systems that use human embryonic kidney (HEK) cells, which have been able to deliver sufficient quantities of protein during the discovery phase. Once a molecule was identified, a stable CHO cell clone would be created for late development and clinical material generation. This approach requires a transition to a new host cell line, incurring additional time and expense when moving from transient to stable clone production.

### The benefits of CHO cells

- CHO cells are the predominant host for biopharmaceutical protein expression, with approximately 70% of licensed biologics manufactured in CHO cells
- Multiple attributes have made CHO cells desirable, including extensive folding, assembly of subunits, and posttranslational modifications such as the critical product quality attribute N-glycosylation
- CHO cells readily adapt to high-density suspension culture in serum-free and chemically defined media
- CHO cells have proven to be a safe host; novel therapeutics manufactured in this cell system are more likely to obtain approval by regulatory agencies

The Gibco™ ExpiCHO™ Expression System enables the use of the same CHO host cell line from discovery through large-scale production, therefore shortening development timelines. This approach enables seamless discovery and production of the desired protein (Figure 1A). The ability to transiently produce CHO cell-derived proteins as early as possible in the biologics development process is highly advantageous, as it minimizes the potential for changes in protein quality and function, which are often observed when moving from HEK to CHO cell lines (Figure 1B).



**Figure 1. Faster development timelines with a single host cell line.**

(A) Unlike traditional systems, the ExpiCHO system offers a direct path to stable clone development by eliminating time-consuming media screening and optimization in biologics process development. (B) Different hosts can glycosylate the same protein in different ways. The same protein overexpressed in CHO-S and HEK293 cells has different glycosylation profiles, which increases the difficulty of transitioning expressed biomolecules from research to development.

## ExpiCHO system components

- **Gibco™ ExpiCHO-S™ cells:** Developed for high protein expression; offered as cells for research use only (RUO) and as cGMP-banked cells for clinical trials.
- **Gibco™ ExpiFectamine™ CHO Transfection Kit:** Highest-efficiency transfection system on the market for CHO cells.
- **Gibco™ ExpiCHO™ Expression Medium:** Supports both high titer and transfection efficiency for transient and stable workflows. Does not require addition of Gibco™ GlutaMAX™ Supplement.
- **Gibco™ ExpiCHO™ Stable Production Medium (SPM):** Supports scale-up of a high-producing stable clone to commercial scale, without requiring any adaptation. Requires addition of GlutaMAX Supplement and is not compatible with transfection.

## ExpiCHO system details

Components of the ExpiCHO system are designed to work together to provide superior performance from transient expression to stable clone production.

- **ExpiCHO-S cells (RUO):** Readily support high-level expression of monoclonal antibodies and antibody-like moieties such as Fc fusion proteins and Fab fragments. The cells have a short doubling time and are adapted for high-density culture with a stable growth and expression profile for over 20 passages.
- **ExpiCHO-S cells (cGMP banked):** Available for clinical trials and commercial licensing without royalty obligations. With the execution of a Commercial Production License, Thermo Fisher Scientific will supply a Cell Line Documentation Package, which includes cell line lineage history, molecular characterization, and adventitious agent testing details for the specific lot of cells purchased, that can be used with an investigational new drug (IND) filing.

- **ExpiFectamine CHO Transfection Kit:** Composed of ExpiFectamine CHO Reagent, ExpiFectamine CHO Enhancer, and ExpiCHO Feed. These three components are optimized to work together in high-density ExpiCHO-S cell cultures.
  - ExpiFectamine CHO Reagent is a highly efficient transfection reagent, enabling use of significantly lower levels of plasmid DNA for expression runs.
  - The addition of enhancer and feed 18–22 hours after transfection contributes to high transfection efficiency.
  - Expression levels >30-fold higher are obtained using the ExpiCHO system as directed, compared to substitution of polyethylenimine (PEI) for the transfection reagent, and using 0.8 µg plasmid DNA per milliliter of culture volume with both.
- **ExpiCHO Expression Medium:** An animal origin-free (AOF), transfection-compatible, high-density growth medium specifically matched to ExpiFectamine CHO Enhancer and ExpiCHO Feed. The medium is chemically defined, so it does not contain any proteins, peptides, or hydrolysates. It contains GlutaMAX Supplement at a concentration of 8 mM and is available in volumes of 1, 10, and 20 L.
- **ExpiCHO SPM:** Chemically defined, AOF medium specifically developed to support high-titer expression of stable ExpiCHO-S clones, particularly at commercial scale. It comes as a liquid as well as in Gibco™ Advanced Granulation Technology™ (AGT™) dry format but does not contain GlutaMAX Supplement. The medium is not compatible with transfection. We offer it as 1 L of the liquid medium and in various sizes of the AGT dry format for reconstitution to 10, 100, and 450 L of liquid. The medium works well with our popular Gibco™ EfficientFeed™ C+ AGT™ Supplement.

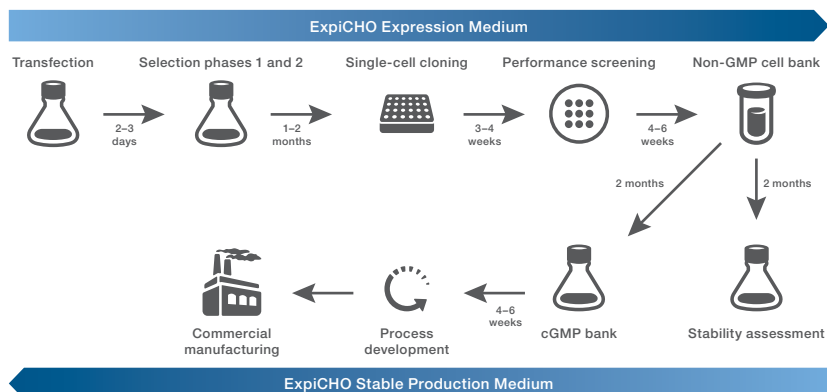
A vector is not provided as part of the platform, but ExpiCHO-S cells are compatible with typical mammalian expression vectors, including CMV promoter-based vectors such as Invitrogen™ pcDNA™3.3-TOPO™ and pcDNA™3.4-TOPO™ vectors.

## Workflow for the ExpiCHO system

For both transient and stable expression workflows, ExpiCHO-S cells are transfected with a mammalian expression vector of choice using the ExpiFectamine CHO Transfection Kit and cultured in ExpiCHO Expression Medium (Figure 2). Selection and clone screening take place in the presence of ExpiCHO Expression Medium, which is also used to bank the cells. The banked clones can be directly thawed into ExpiCHO SPM for subsequent procedures. These include stability assessments for the banked clones, any subsequent cell banking of stable clones under cGMP procedures, and large-scale bioprocessing for the commercial manufacture of biomolecules such as monoclonal antibodies. For detailed protocols for transient protein expression and stable clone production, please see the user guides [1,2].

### Workflow details

- 1. Transfection:** Thawed ExpiCHO-S cells are passaged at least 3 times and transfected with an expression vector using the ExpiFectamine CHO Transfection Kit.
- 2. Selection phases 1 and 2:** 48 hr after transfection, ExpiCHO-S cells are selected with an antibiotic concentration that was determined using a kill curve. After completion of selection phase 1, selection phase 2 is started by increasing the antibiotic concentration between 2x and 5x. Selection phase 2 is completed when the recovered cells show viability of at least 90%. The recovered cells or cell pools are cryopreserved and tested in a productivity assessment assay, which tests for cell viability, cell density, and protein expression.
- 3. Single-cell cloning and scale-up of single clones:** The cryopreserved cells are thawed, passaged, and subjected to limited dilution cloning (LDC) to isolate single clones from the cell pool. (No antibiotics are used at this stage.) A single cell in each well of a 96-well plate grows into a colony. Once the colonies in the 96-well plates are confluent, they are passaged into 24-well plates and, from there, into 6-well plates. The clones in the 6-well plates are then passaged or expanded further and cryopreserved, and a productivity assessment is performed to identify the top 15 to 40 clones (primary screen). The clones are expanded and passaged twice in 30 mL cultures, cryopreserved, and tested for productivity to identify new top clones (secondary screen). The clones from the secondary screen are thawed and passaged in 30 mL cultures 2 to 5 times, cryopreserved, and tested for productivity to identify the lead clones (tertiary screen).
- 4. Non-cGMP cell bank:** Clones from the performance screening step are used for stability testing and additional cell banking.
- 5. Stability assessment:** Typically, the top 16 clones of the tertiary screen will undergo a stability assessment. Here the clones are tested for 60 generations to confirm that the protein expression level does not decrease significantly. Top clones of the primary or secondary screen can also be tested. The top clones are thawed and cultured in ExpiCHO SPM. Clones that fulfill the stability assessment criteria are chosen for the next steps.
- 6. cGMP-banked ExpiCHO-S clones:** These clones are cryopreserved under cGMP procedures and banked as cGMP clones. They have been selected and characterized as previously described.
- 7. Process development:** cGMP-banked ExpiCHO-S clones are thawed, cultured, and expanded in ExpiCHO SPM. The clones are tested for protein expression in a bioreactor at small, medium, and large scales.
- 8. Commercial manufacturing:** cGMP ExpiCHO-S clones are grown in large bioreactors to produce the protein of interest, such as a monoclonal antibody.

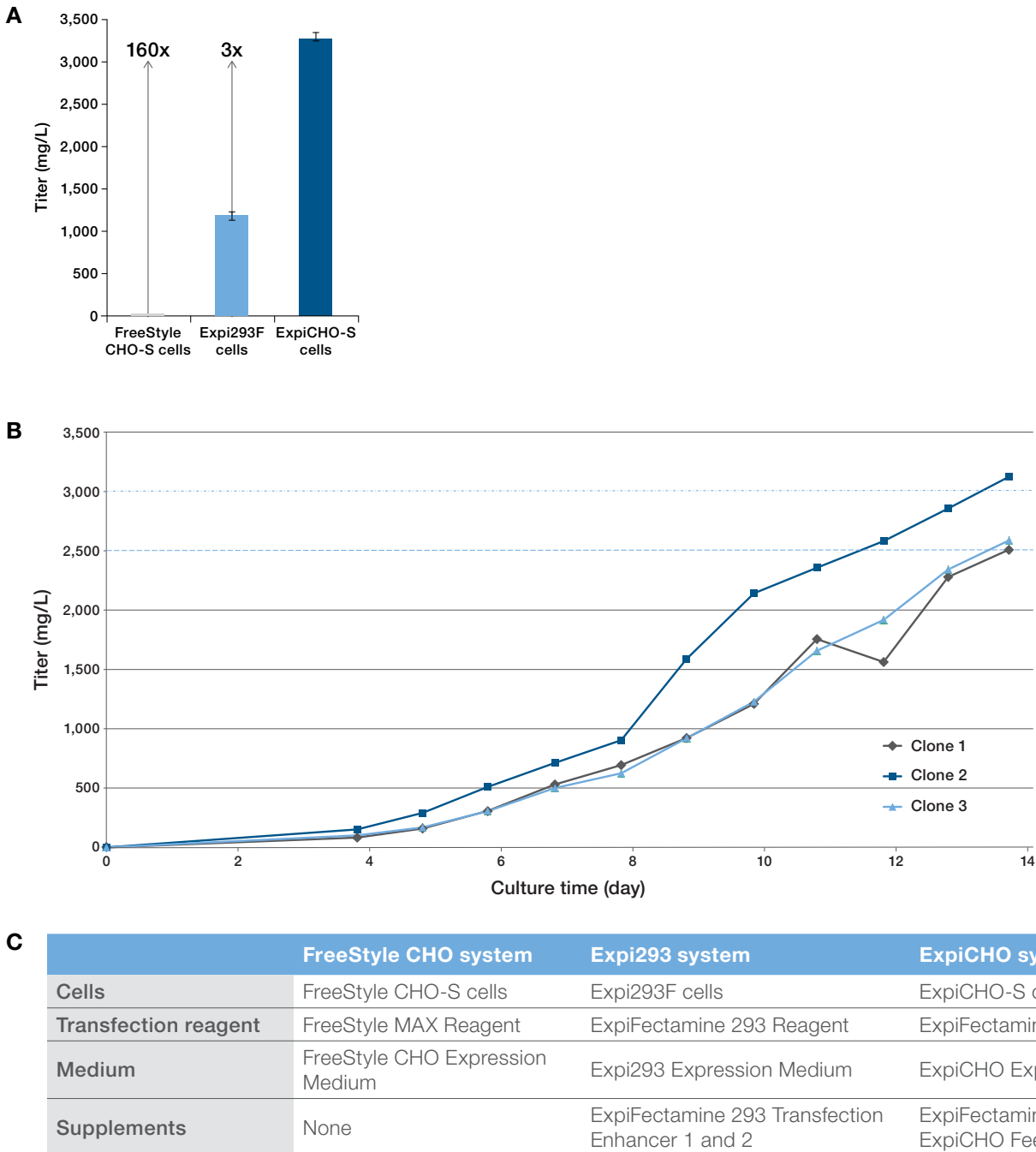


**Figure 2. Overview of stable clone production using the ExpiCHO system.** This workflow demonstrates the ease of transition from transient to stable protein production.

## Results

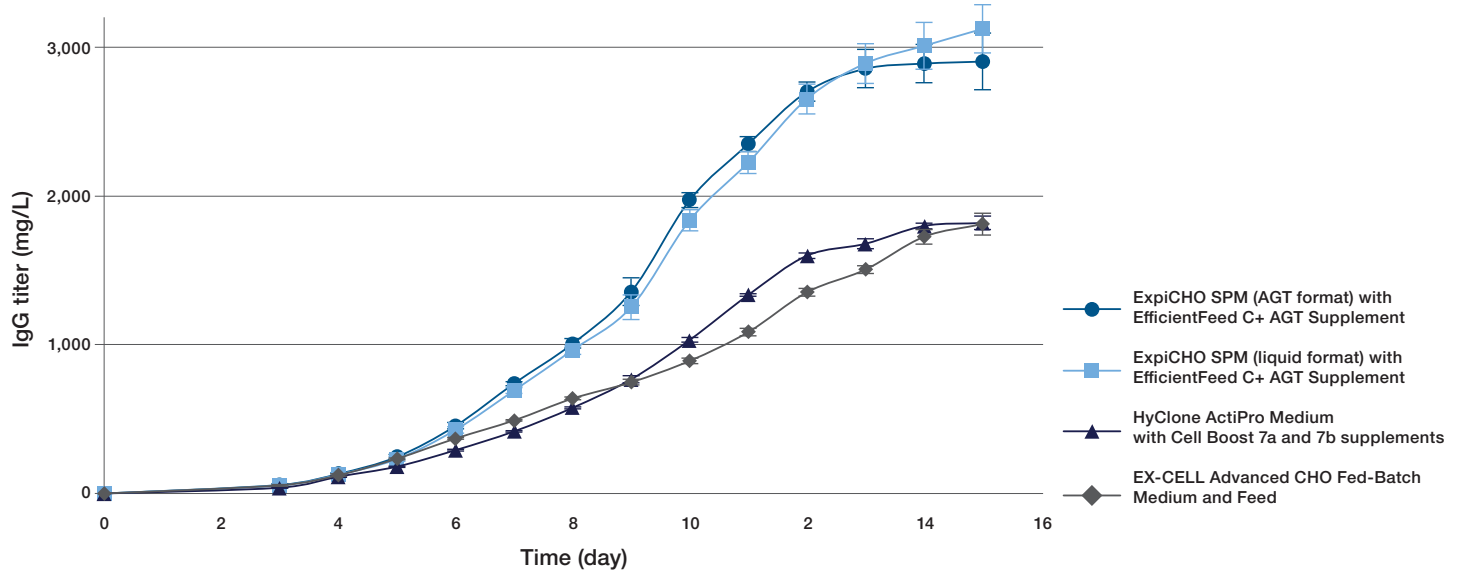
ExpiCHO-S cells support the transient production of recombinant proteins to exceed yields from Gibco™ FreeStyle™ CHO-S and Expi293F™ cells. FreeStyle CHO-S, Expi293F, and ExpiCHO-S cells were transiently transfected with the same vector using their respective transfection reagents and protocols (Figure 3A). The ExpiCHO-S cells expressed approximately

3 g/L human IgG, demonstrating approximately 160x and 3x higher titers than the FreeStyle CHO-S and Expi293F cells, respectively. A number of high-producing clones were selected from stably transfected ExpiCHO-S cells, and then grown in ExpiCHO SPM (Figure 3B). There is good agreement between titers from transient and stable expression of the same monoclonal antibody.

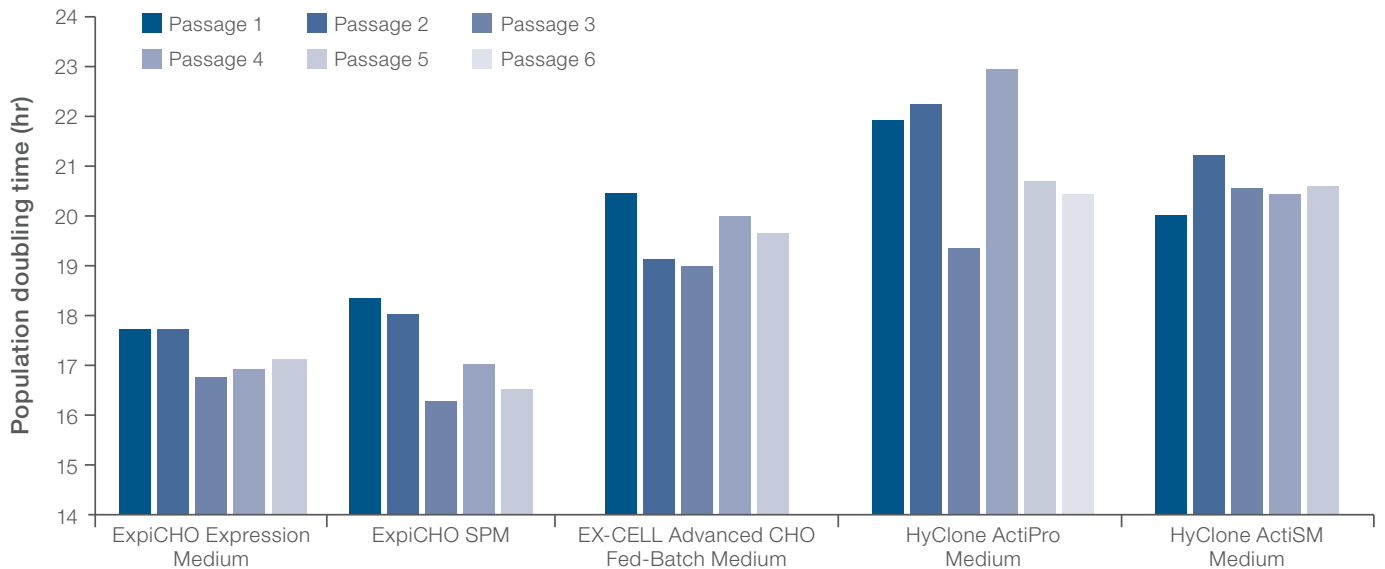


**Figure 3. High IgG titers in transient and stable expression. (A)** Titers of the same human IgG transiently expressed in different cell lines are shown. **(B)** Stably transfected ExpiCHO-S clones were assessed for IgG titer. **(C)** Reagents developed for each system were used for protein expression.

ExpiCHO SPM, in either liquid or AGT format, was able to support ExpiCHO-S cells in generating the highest titers in fed-batch cultures compared to media formulations from other vendors (Figure 4). In addition, there was consistent growth performance of ExpiCHO-S cells in both ExpiCHO Expression Medium and ExpiCHO SPM (Figure 5). Growth performance as measured by doubling time was superior to that in alternative media.



**Figure 4. ExpiCHO SPM produces superior titers in fed-batch cultures.** ExpiCHO Expression Medium was used for stable transfection and clone selection. All clones were transitioned to their respective media without adaptation. ExpiCHO SPM (AGT or liquid format) with EfficientFeed C+ AGT Supplement supports the highest IgG titer.



**Figure 5. ExpiCHO-S cells have the shortest cell doubling time in ExpiCHO media compared to other systems.** ExpiCHO-S cells were stably transfected in the presence of ExpiCHO Expression Medium. Clone selection was performed in the same medium. Stable ExpiCHO-S clones were transitioned to ExpiCHO Expression Medium, ExpiCHO SPM, EX-CELL™ Advanced™ CHO Fed-Batch Medium, HyClone™ ActiPro™ Medium, or HyClone™ ActiSM™ Medium, and passed up to 7 times.



## Conclusion

Achieving a streamlined workflow for the bioproduction process has traditionally been challenging due to the use of different cell lines for the discovery and manufacturing phases. We have shown that the ExpiCHO system offers a simplified and scalable workflow that eliminates cumbersome media screening and clone adaptation during the development process. Transient and stable expression with the ExpiCHO system show high protein levels and antibody titers. Stable ExpiCHO-S clones easily transition from EpiCHO Expression Medium to ExpiCHO SPM, which are both compatible with EfficientFeed C+ AGT Supplement. ExpiCHO SPM is offered in dry AGT format, enabling fast scale-up of stable clones that originate from cGMP-banked ExpiCHO-S cells and are used for commercial manufacturing.

The ExpiCHO system helps accelerate protein expression by bringing together cells grown to high densities, Gibco™ cell culture media, and Invitrogen™ transfection technologies. This combination of advanced solutions helps streamline development of protein-based drugs and vaccines, enabling faster production of potentially life-changing biologics.

## References

1. User guide: ExpiCHO Expression System. Pub. No. MAN0014337, Rev. D.0.
2. User bulletin: Creation and scale-up of a stable cell line using ExpiCHO products. Pub. No. MAN0017764, Rev. 2.0.

“The ExpiCHO system helps speed up our developmental timelines by eliminating the need to have different hosts for transient versus stable expression. This eliminates a key quality risk when we transition from research into development.”

– Neeraj Kohli  
Associate Director at Merrimack Pharmaceuticals

“The ExpiCHO system is so easy it takes less work than making proteins in *E. coli*. The scalability of the system is fantastic. I can express 30 different mAbs simultaneously, and then pick the ones I like and scale up to grams. The ExpiCHO system is a revolutionary lifesaver for us.”

–Arjen van den Berg, PhD, Senior Scientist  
Cellular and Molecular Medicine  
University of California, San Diego

Find out more at [thermofisher.com/expicho](https://thermofisher.com/expicho)

**ThermoFisher**  
SCIENTIFIC