

# High-Level Transient Recombinant Protein Production in CHO Suspension Cells using Corning® 5L Erlenmeyer Flasks

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## Application Note

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### Introduction

Mammalian cells are a well-established system for the expression of biologically active recombinant proteins and antibodies that possess appropriate post-translational modifications.<sup>1</sup> Stable expression technologies have yielded kilograms of protein from mammalian cells.<sup>2</sup> However, some major disadvantages of stable expression technology are the time, resources, and costs associated with the generation of a high-producing cell line.<sup>3</sup> Therefore, there is a need for more rapid and less expensive approaches for recombinant protein production. Transient gene expression is a popular method for efficient production of recombinant proteins in mammalian cells. With optimization and scale-up, transient transfection in mammalian cells can improve the throughput and speed of production of functional human proteins.<sup>3</sup>

Chinese hamster ovary (CHO) cell suspensions are well-suited for high-yield production of recombinant proteins. The Gibco ExpiCHO™ Expression System uses a CHO-S cell line, expression media designed for high-density transfection, expression enhancers, feeds, and a high-efficiency transfection reagent to maximize protein expression levels.<sup>4</sup>

The Corning 5L PETG Erlenmeyer flask is designed for scale-up suspension cultures with space efficiency in mind, thus supporting CHO cell line expansion and recombinant protein expression. The PETG material is free of 3,5-bisnitro-BPA leachate which can inhibit cell growth providing optimized culture conditions for sensitive suspension cell lines. The 5L shape has also been optimized for increased gas exchange compared to the more traditional Erlenmeyer flask designs. In this study, we describe a method for the transient transfection of CHO cells in Corning 5L Erlenmeyer flasks, which allow for enhanced recombinant protein production. We also compared the performance of the Corning 5L PETG Erlenmeyer flask to 5L polypropylene (PP) flasks from a comparable brand.

### Materials and Methods

#### Cell Culture

ExpiCHO-S cells (Thermo Fisher Cat. No. A2910001) were cultured in ExpiCHO Expression Medium (Thermo Fisher Cat. No. A2910001) in a humidified 8% CO<sub>2</sub> incubator at 37°C. Cultures were maintained in different size flasks at the indicated rpm on shakers with a 50 mm throw. ExpiCHO-S cells were passaged at a density of approximately 4 x 10<sup>6</sup> to 6 x 10<sup>6</sup> viable cells/mL (i.e., early log phase growth), typically every 3 to 4 days.

### Large-scale Transfection

The plasmid DNAs used throughout this study were a derivative of an expression plasmid (Thermo Fisher Cat. No. A14662). The expression plasmids contained light chain cDNAs encoding rabbit IgG antibody. Transfection grade plasmid DNA was isolated using a plasmid purification kit following the manufacturer's protocol.

One microgram of plasmid DNA per milliliter of transfection culture medium was used for ExpiCHO transfections, and transfections were performed on a 2L scale in Corning 5L Erlenmeyer flasks with plain bottom (Corning Cat. No. 431284) and 5L Polypropylene (PP) flasks from a comparable brand. Cultures were maintained at 80 rpm on shakers with a 50 mm throw. The day prior to transfection, cells were split to 4 x 10<sup>6</sup> cells/mL. After 24 hours, stocks had a density of 8 x 10<sup>6</sup> cells/mL and were diluted to 6 x 10<sup>6</sup> cells/mL with fresh medium. ExpiCHO transfections were performed using the ExpiCHO Expression System Kit (Thermo Fisher Cat. No. A29133) according to the manufacturer's Max Titer protocol.

### Protein Yield

To establish a time course of cell titers, samples of transfected cultures were taken at indicated timepoints. Cells were counted, and cell viability was assessed using the Trypan blue exclusion assay. An additional 2 mL sample was collected and centrifuged at 2000 x g for 15 minutes at room temperature, after which the supernatant was transferred to a fresh tube for protein quantitation. Protein concentration was quantified using ELISA and SDS-PAGE.

### Results and Discussion

Figure 1 and Figure 2 show ExpiCHO-S cell density and viability post-transfection in the Corning 5L Erlenmeyer flask. The cell culture reached a maximum cell density of 1.1 x 10<sup>7</sup> cells/mL 2 days post-transfection. By Day 3, cell density had decreased to 7.6 x 10<sup>6</sup> cells/mL. Four days after transfection, cell density slowly decreased to 5 x 10<sup>6</sup> cells/mL. Cell viability was maintained above 80% for up to 12 days after transfection.

ExpiCHO-S cells were transfected with plasmids, and protein expression was determined at 2, 6, and 12 days post-transfection (Figure 3). Six days post-transfection, the protein expression levels were significantly higher in the Corning 5L Erlenmeyer flask than in the comparable brand 5L Erlenmeyer flask. Protein expression reached optimal yields of 120 mg/L in the Corning 5L flask and 55 mg/L in the comparable brand 5L flask at Day 12 post-transfection.

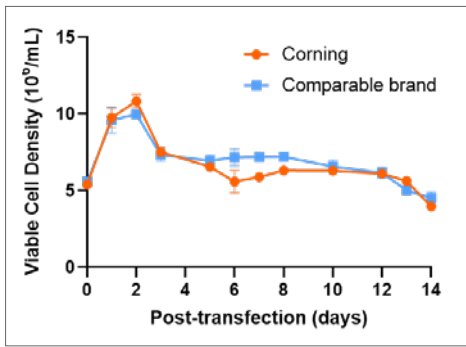


Figure 1. ExpiCHO-S cell density in the Corning and the comparable brand 5L Erlenmeyer flask. Data are represented as mean  $\pm$  SD.

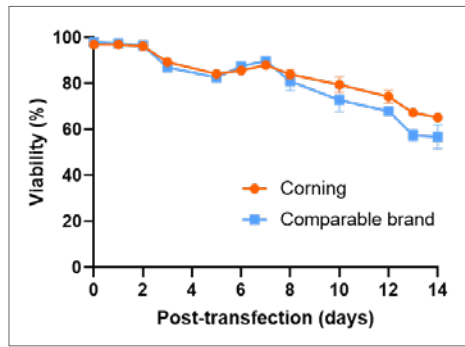


Figure 2. ExpiCHO-S cell viability in the Corning and the comparable brand 5L Erlenmeyer flask. Data are represented as mean  $\pm$  SD.

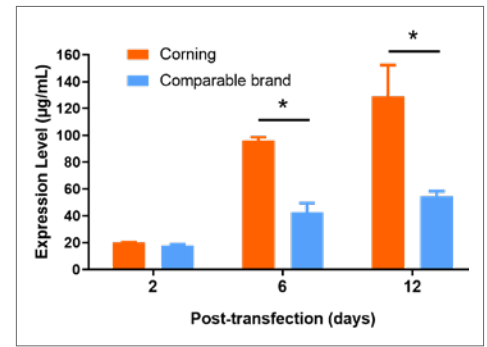


Figure 3. Expression of recombinant protein in the Corning and the comparable brand 5L Erlenmeyer flasks. After 6 days of growth, the vessel from the comparable brand exhibited statistically lower cell densities compared to the Corning 5L Erlenmeyer flask. Data are represented as mean  $\pm$  SD. \* $p < 0.05$ .

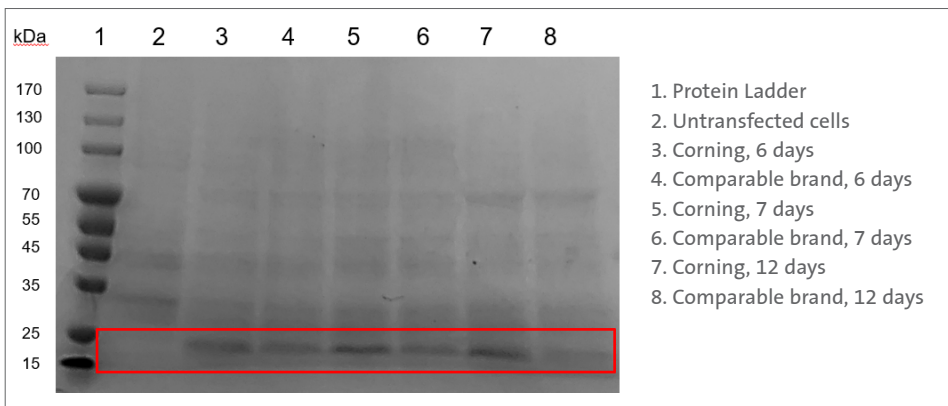


Figure 4. Coomassie-stained SDS-PAGE gel comparing target protein expression between the Corning and the comparable brand 5L Erlenmeyer flasks.

A Coomassie-stained SDS-PAGE gel (Figure 4) showed the target protein band at approximately 25 kDa. The band density indicated that the protein expression in the Corning 5L Erlenmeyer flask was higher than that in the comparable brand 5L Erlenmeyer at 12 days post-transfection.

## Conclusions

- ▶ The Corning 5L PETG Erlenmeyer flask can support high-density ExpiCHO-S transient transfection.
- ▶ Over 100 mg/L of recombinant protein was obtained in the Corning 5L Erlenmeyer flask 12 days after transfection. This yield was 2-fold higher than the yield of the equivalent 5L flask from a comparable brand.
- ▶ The higher level of recombinant protein expression is likely due to the optimized design of the Corning Erlenmeyer 5L flask whose higher liquid surface area to volume ratio results in better culture aeration and mixing.

## References

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4. Thermo Fisher Scientific Poster: Strategies for high-titer protein expression using the ExpiCHO and Expi293 transient expression systems.

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