

Multi-Gram Scale Antibody Production Using CHO Cell Transient Gene Expression via Flow Electroporation

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Abstract

In recent years researchers have turned to transient gene expression (TGE) as an alternative to CHO stable cell line production for early stage antibody development (1,2). Despite advances in transfection methods and culture optimization, the majority of CHO-based TGE systems produce insufficient antibody titers (low mg/L level) for full use within the biotherapeutic development pipelines (3-8). MaxCyte electroporation provides a universal means of fully scalable, highly efficient CHO-based TGE for the rapid production of gram level quantities of antibodies without the need for specialized reagents, expression vectors, or engineered CHO cell lines. The high productivity of MaxCyte-driven TGE allows for its use in early phase candidate identification as well as for generating the gram level antibody quantities needed for later stage pharmacology, stability, and manufacturability studies. In this technical note, we present data demonstrating the reproducibility, scalability, and antibody production capabilities of MaxCyte electroporation. Secreted antibody titers routinely exceed 400 mg/L and can exceed **2.7 g/L** following optimization, thereby enabling multi-gram antibody production from a single, CHO cell transfection. In addition, we present data showing the use of MaxCyte electroporation for the rapid generation of high-yield stable CHO cell lines to bridge the gap between early and late stage antibody development activities.

Introduction

Mammalian cells, and in particular, CHO stable cell lines continue to be the system of choice for biomanufacturing of clinical-grade biotherapeutics. During early stage biotherapeutic development, however, hundreds of candidates may be under evaluation and creation of stable cell lines for each candidate would require large investments of time and money. As a result, industry has looked to transient gene expression (TGE) to rapidly screen large numbers of antibodies or antibody-like molecules early in the development process to identify promising candidates for further evaluation (1,2).

Initial CHO-based TGE activities within the biopharmaceutical community were limited by poor transfection efficiencies, cell viabilities, and production of insufficient quantities of antibodies. This led to the use of HEK-based transient systems, which historically have higher transfection efficiencies and antibody production capabilities. Multiple reports, however, have shown that there are differences in the manufacturability, affinity, and efficacy of antibodies produced in HEK cells compared to those produced by stably transfected CHO cells (3). In response, there is strong interest in developing CHO-based TGE systems that can rapidly and efficiently produce gram quantities of antibodies, eliminating the need to use HEK-based expression systems as a surrogate.

A variety of CHO cell transient transfection methods have been published including systems based on engineered CHO cells, unique expression constructs, specialized reagents, or proprietary technologies available through licensing or services (1,3-8). These systems as well as highly optimized protocols for lipids and polyethylenimine (PEI) have varying levels of reproducibility, scalability, and cost effectiveness, and in general produce antibody titers from

10–100 mg/L for typical IgG. These systems typically have even lower expression levels for difficult-to-express molecules such as bispecific antibodies. A transient CHO system that easily scales from milligram to multi-gram production capabilities would be advantageous and enable the use of CHO TGE as a tool for both early and mid-stage biotherapeutic development activities.

MaxCyte's proprietary flow electroporation technology is a universal transient transfection platform for rapid, high quality cell transfection. Flow electroporation combines superior performance, broad applicability, and ease of use with the capacity to transfect up to 2E11 cells in under 30 minutes. This technology is fully scalable and achieves multi-gram CHO antibody production without requiring specific expression constructs, adapted CHO lines, specialized reagents, or media additives. Secreted

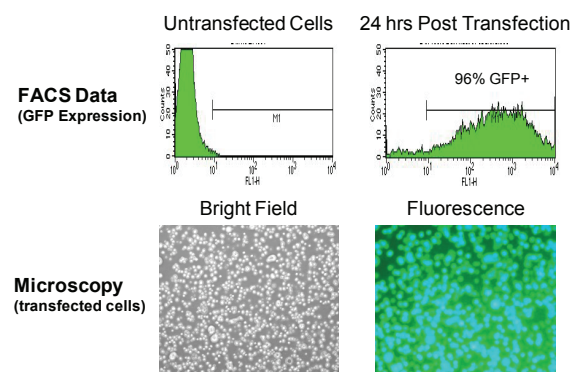


Figure 1: Greater than 95% CHO Cell Transfection Efficiency and Cell Viability Using MaxCyte Transient Transfection. CHO-S cells were transfected with a plasmid encoding green fluorescent protein (2 µg DNA/1E6 cells) using small-scale, static electroporation on the MaxCyte STX. GFP expression and viability were measured by flow cytometry (FACS) 24 hours post electroporation.

antibody titers are routinely greater than 400 mg/L and can exceed 2.7 g/L with optimization. Additionally, MaxCyte electroporation is a powerful tool for accelerating the generation of high-yield stable CHO cell lines, providing for maximum efficiency in the progression to late stage development and biomanufacturing. Thus, flow electroporation offers a single, cost-effective means of CHO cell antibody production throughout biotherapeutic development pipelines.

MaxCyte Transient Transfection

MaxCyte offers two benchtop transfection systems based on flow electroporation: the MaxCyte STX[®] Scalable Transfection System and the MaxCyte VLX[®] Large Scale Transfection System. Both systems are pre-loaded with protocols for simple push-button use and support the transfection of a variety of cell types, including cells commonly used for protein production such as CHO, HEK, NS0, Vero, and insect cells. The MaxCyte STX has the flexibility to transfect from as few as 5E5 cells in seconds using small-scale, static electroporation up to 2E10 cells in less than 30 minutes using flow electroporation. The MaxCyte VLX is designed for extremely large-scale transfections with the capacity to transiently transfect up to 2E11 cells within 30 minutes. The large capacity and high performance of both the MaxCyte STX and VLX allow these systems to support multi-gram production of antibodies and proteins from a single 30-minute transfection.

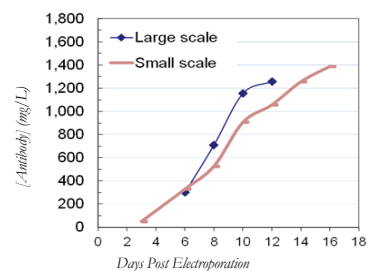
High Efficiency CHO Cell DNA Loading

Transient transfection of CHO cells has historically resulted in lower transfection efficiencies than other cell types used for protein expression such as HEK cells. MaxCyte electroporation enables high efficiency, high viability CHO cell transient transfection without the need for costly transfection reagents. CHO-S cells transiently transfected with a plasmid encoding green fluorescent protein display greater than 95% transfection efficiency and cell viability (Figure 1). In addition to CHO-S cells, MaxCyte electroporation has been used to transfect a variety of adherent and suspension CHO cell lines, including CHO-K1 and CHO-DG44 (data not shown).

High Titer CHO Antibody Production

The amount of antibody required throughout the biotherapeutic development process varies from low milligram to multi-gram quantities. Ideally, the transient transfection system of choice will have the scalability to simply and rapidly produce the full range of antibody quantities needed. Other CHO transient transfection methods, including various PEI and lipid-based reagents and protocols, report antibody titers ranging from 2 to 150 mg/L upon full optimization (3-8). These methods can be limited by a range of factors such as reproducibility, scalability, functional feasibility, and cost considerations. In contrast, MaxCyte flow

Rapid, Gram Scale Antibody Production



	Culture Volume	EP Volume	# of Cells	[IgG]	Total IgG Produced
Small Scale	20mL	0.4mL	8E7	1.40 g/L	28mg
Large Scale	2.8L	50mL	1E10	1.22 g/L	3.42 g

Figure 2: High Titer Antibody Production with Seamless Scalability. 8E7 or 1E10 CHO-S cells were transfected with an antibody expression plasmid (2 µg DNA per 1E6 cells) via small-scale (static) or large-scale (flow) electroporation on the MaxCyte STX. Cells were plated at 6E6 cells/mL post electroporation. 1mM sodium butyrate was added to cultures and the temperature lowered to 32°C 24 hours post electroporation. Cultures were fed daily with a media optimized for antibody production. Total IgG was measured using ELISA on various days post transfection.

electroporation can scale to produce multi-gram quantities of antibody within two weeks of CHO transient transfection, with titers generally more than 400 mg/L, which can be further increased upon optimization.

Antibody Production Using Flow Electroporation

MaxCyte large-scale transfection studies demonstrate the ability of flow electroporation to rapidly produce grams of antibody using CHO transient gene expression (Figure 2). Antibody titers of greater than 1.2 g/L were detected 12 days after transfection of 1E10 CHO-S cells using post transfection culture conditions optimized for antibody productivity. Greater than 3 grams of antibody were produced from less than 3 Liters of CHO culture. The MaxCyte VLX has the capacity to transfect 2E11 cells, which would equate to production of more than 50 grams of antibody within two weeks of transfection using a single transient transfection.

Comparison studies of small- and large-scale electroporation illustrate the consistent and reproducible performance of MaxCyte technology (Figure 2). These studies also highlight the ability to scale up MaxCyte transfection without the need for further optimization. Comparable cell viabilities and antibody titers were observed upon small- and large-scale transfection using the same electroporation parameters and general cell handling methods. Both transfections led to increasing antibody titers that peaked at greater than **1.2 g/L** during the 14-day study, demonstrating the magnitude and extended period of antibody expression following cell electroporation. Additional studies have confirmed high levels of cell productivity for over 21 days, thus increasing the production potential of a single transfection run (Figure 3).

Normal Antibody Characteristics

Antibodies produced via MaxCyte transient transfection were characterized and compared to those produced by a stably transfected cell line (Figure 4). CHO cells were transiently transfected with an antibody expression plasmid and secreted antibody samples (S) collected for analysis via electrophoresis. The same antibody was produced via a stable CHO cell line and used as a reference (R) during analysis. Similar to previous reports (6,7), no differences between transiently and stably produced antibodies from CHO cells are observed when examined via reducing and non-reducing SDS-PAGE, as well as Isoelectric Focusing. This supports the use of CHO-based transient gene expression via MaxCyte electroporation during the early identification of relevant therapeutic antibodies.

Increased Yield and Laboratory Productivity

Significant efforts have been made to increase CHO cell antibody productivity from both transient and stable expression systems. A range of factors have been identified that affect productivity including the use of specific CHO cell lines and various cell culture parameters such as cell density, feeding conditions, media additives, and culture temperature (2,3,5,7-9). We examined a variety of post transfection parameters and found the combination of a hypothermic temperature shift, the use of sodium butyrate, and nutrient feed significantly improved antibody titers with optimum titers of greater than 1.2 grams/L (Figure 5 and data not shown). While these specific parameters increased productivity in our laboratory, MaxCyte technology is universal in nature, providing researchers the flexibility to use their expression system and cells of interest, as well as lab-specific cell handling methods.

Effects of Media Composition and Feeding Strategy

Cell growth, nutrient depletion, and secreted protein titers are interrelated and are influenced by media formulation (9). To examine these effects, CHO cells were transfected,

Multi-Gram Scale Antibody Production by TGE

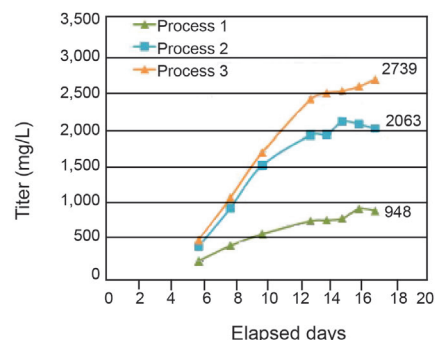


Figure 3: Transient Expression of hlgG1 Antibody in MaxCyte EP Transfected CHO-S cells. The same transfected cells were in different production processes. Further optimized process (process 3) can reach 2.7 g/L as a fed batch. Titer was verified by both ELISA and Protein A capture assays.

Secreted Antibody Analysis

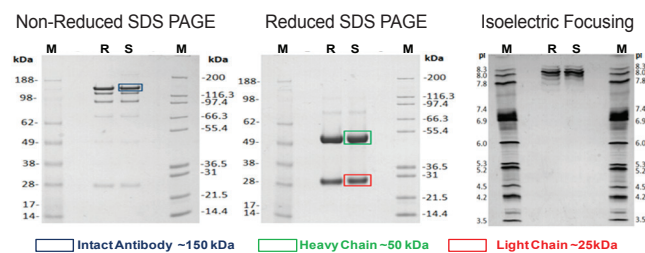


Figure 4: Similarity of Antibodies Produced from Stably and Transiently Transfected CHO Cells. CHO suspension cells were transfected by static electroporation with a plasmid encoding IgG heavy and light chain sequences. Secreted antibodies from electroporated cells (S) and a reference stable cell line (R) were examined by reducing and non-reducing SDS-PAGE and Isoelectric Focusing. Data courtesy of NovImmune.

resuspended in a media, and cultured using one of three feed strategies. Overall, we found that the variant feed strategy (Process 3) in which nutrients were replenished more frequently, generated antibody titers approximately threefold higher than Process 1. By optimizing the feed strategy we achieved an antibody titer of **2.7 grams/L**. These results demonstrate the ability to maximize antibody production by optimizing post transfection cell culture nutrients.

Post Transfection Cell Density vs. Antibody Production

Increasing laboratory productivity is a combination of maximizing yield while minimizing labor and material resource requirements. We examined the antibody production capabilities of transfected CHO cells cultured at high cell densities (using unoptimized feeding conditions) in an effort to streamline downstream processing and minimize media usage. Transfected cells inoculated at $1E7$ cells/mL produced significantly higher antibody titers than cells inoculated at $6E6$ cells/mL (Figure 5). High density cultures had secreted antibody titers exceeding 800 mg/L within 15 days of transfection.

Transfection & Cell Processing Scale Up

MaxCyte electroporation enables straightforward scale up of the transfection process without requiring re-

Titers >800 mg/L Upon Higher Cell Density Inoculation

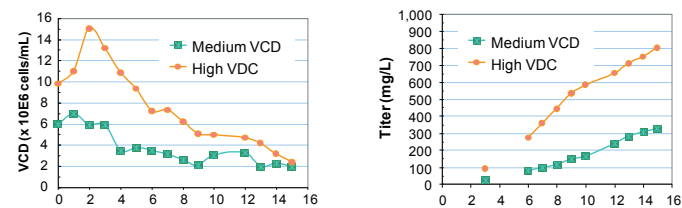


Figure 5: Post Transfection Inoculation at Higher Cell Densities Increases Antibody Titers. $1E10$ CHO-S cells were transiently transfected with an antibody expression plasmid ($1 \mu\text{g DNA}/1E6$ cells) via flow electroporation. Post electroporation, cells were inoculated into two shake flasks at densities of $6E6$ or $1E7$ cells per mL.

optimization of transfection conditions. The large capacity of MaxCyte electroporation also allows scale up of post transfection antibody production processes. CHO cells transfected using either static or flow electroporation and cultured in 250 mL shake flasks using unoptimized feeding conditions lead to secreted antibody titers of approximately 400 mg/L at day 14 post transfection, illustrating the scalability and reproducibility of MaxCyte technology (Figure 6). Additionally, CHO cells transfected via flow electroporation and cultured in larger 3-L shake flasks maintained similar cell viability with antibody titers greater than 400 mg/L, demonstrating the ability to scale up culture vessels post transfection for bulk processing. Studies using wave bags confirm the capacity of vessel scale up for manufacturing purposes (data not shown).

Antibody Titers Maintained During Process Scale Up

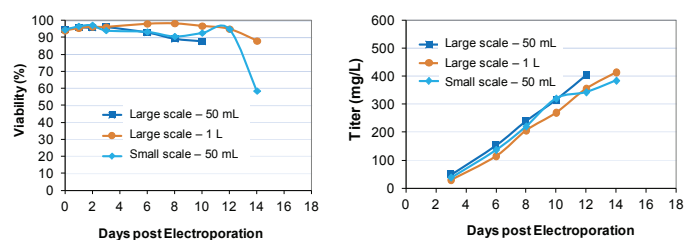


Figure 6: Titers Maintained Upon Transfection and Culture Vessel Scale Up. 1E10 or 2.4E8 CHO-S cells were transfected with an antibody expression plasmid (1 µg DNA/1E6 cells) using flow or static electroporation, respectively. Cells transfected via static EP were inoculated into 50 mL of media in a 250 mL shake flask. Cells transfected via flow EP were split and inoculated into 1 L of media in a 3-L shake flask or into 50 mL of media in a 250-mL shake flask. Starting cell densities were identical in all three flasks.

Production Scale Up Using Stable Cell Lines

The ability to produce gram level quantities of antibodies using flow electroporation extends the applicability of TGE. Stable CHO cell lines remain the regulatory standard for manufacturing of clinical-grade biotherapeutics. Thus, later stage antibody development invariably entails generation of stable cell lines. Recently, researchers have begun looking to antibody production by CHO stable pools to bridge the gap between antibody development and biomanufacturing (1,10). In addition to its use for TGE, flow electroporation is an ideal method of rapidly creating stable pools as well as generating high-yield stable cell clones.

Stable Cell Line Generation

CHO cells were transfected using MaxCyte electroporation to create a stable pool for use in generating stable cell lines. Within six weeks of the initial transient transfection, approximately 500 CHO cell clones were generated and screened. Clone 17 was identified as a top performer and displayed sustained productivity as high as 60 pg/cell/day over a 21-day production period with a final antibody titer of 5.7 g/L (Figure 7).

Flow Electroporation for Stable Cell Line Generation

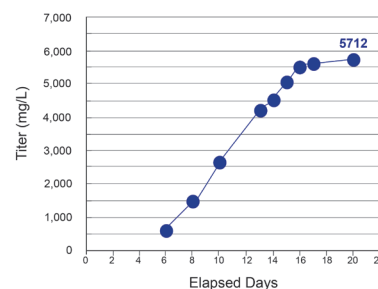


Figure 7: A Fed Batch of a Stable Clone. A stable pool was generated within two weeks of electroporation, and 479 clones were screened following limited dilution cloning. The top clone was selected for production within 6 weeks post transfection. The production was carried out in the shake flask as a fed batch. At day 21, productivity can reach 5.7 g/L and results were verified by both ELISA and Protein A capture assays.

Conclusions

MaxCyte-driven TGE facilitates the identification of quality candidates and their rapid progression through pre-clinical studies while simultaneously streamlining the creation of high-yield stable CHO cell lines for scale up to clinical-grade biomanufacturing. Flow electroporation is a proven means of rapid, high performance CHO cell transient transfection. High transfection efficiencies and cell viabilities enable secreted antibody titers that routinely exceed 400 mg/L within about two weeks of transfection and can exceed 2.7 g/L with optimization. Separately or in parallel, the same process can be used to generate stable pools with subsequent selection of high producing clones with an antibody titer greater than 5.7 g/L. MaxCyte transient transfection has unmatched quality, flexibility, and scalability, yielding gram to multi-gram quantities of antibodies and fulfilling the complete range of biotherapeutic development activities.

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