MaxCyte®
FLOW TRANSFECTION SYSTEMS™

Scalable Protein Production Using Flow Electroporation

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Abstract

Many transient gene expression (TGE) methods produce insufficient protein quantities for full use within biotherapeutic and vaccine development pipelines. MaxCyte's proprietary flow electroporation provides a universal means of highly efficient TGE for the rapid production of large-scale quantities of proteins, antibodies, antibody-like molecules, virus-like particles (VLPs), and vaccines. In addition, the platform can be used for generation of stable pools and stable cell lines that can greatly streamline biotherapeutic and vaccine development. Flow electroporation (co)transfects a wide range of cells, including mammalian and insect cells, with DNA and RNA and combines superior performance, broad applicability, and ease of use with the capacity to transfect up to 2E11 cells in under 30 minutes. This technical note reviews the key features of MaxCyte transfection, including cell type flexibility, scalability, consistency, and high-yield protein production that makes this an ideal TGE platform for use in early-phase candidate identification as well as for generating the gram-level antibody quantities needed for late-stage pharmacolgy formulation, stability, and process and purification development. Case studies are presented for gram-scale IgG production in CHO cells, diabody production, and insect cell VLP production. Comparisons with baculovirus expression and lipid-based transfections are shown.

Introduction

Small molecule, biotherapeutic, and vaccine development activities frequently require the expression of a variety of proteins ranging from simple recombinant proteins to more complex antibodies, antibody-like molecules, and virus-like particles (VLPs). Stable cell lines have been the standard for protein production for over two decades; however, their creation is a costly, time consuming, and labor-intensive process and may not be possible for all applications. In response, researchers have looked to transient gene expression (TGE) as a means of more rapid, cost-effective protein production, particularly during early development and preclinical stages (1). While TGE generally offers a means of more rapidly expressing proteins, not all transient expression methods meet the requirements for larger scale, gram-level protein production needed throughout the development pipeline.

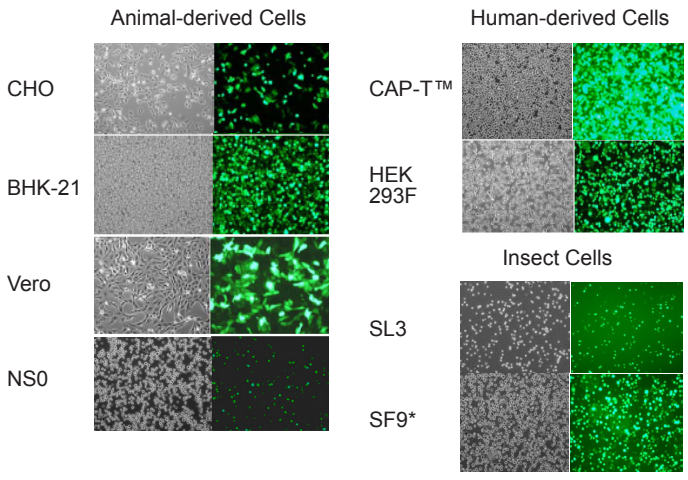


Figure 1: High Efficiency Transfection of Cell Types Commonly Used for Protein Production. *Various cells were transfected with 2 µg/1E6 cells of pGFP DNA using the appropriate MaxCyte STX protocol. Cells were examined for GFP expression using fluorescence microscopy 24 hrs post electroporation. *SF9 cells examined at 72 hrs post electroporation.*

Transient transfection technologies have evolved from simple chemical carriers such as DEAE-dextran, calcium phosphate, and PEI into sophisticated, highly engineered methodologies such as lipid-based reagents, viral-mediated delivery, and electroporation. These newer transfection methods generally provide enhanced performance and broader applicability when compared to earlier methods, allowing for transfection of mammalian cells, a more difficult subset of cells to transfect, but of high interest during biotherapeutic and vaccine development. Key factors to consider when choosing a transient, protein production system range from cell viability, transfection efficiency, and reproducibility to more practical considerations including ease of use, system flexibility, and resource requirements, as well as the overall cost-effectiveness of the system.

Chemical carriers, while affordable, are prone to high levels of variability and lower expression levels even when using optimized protocols. In contrast, lipid-mediated transfection, virus delivery, and electroporation are more reproducible but differ significantly in scalability, time requirements, and cost. Second generation lipid-based

technologies have relatively high transfection efficiencies and the ability to transfect a range of cells, but require re-optimization during scale-up and can become cost prohibitive. Viral delivery methods are scalable and can achieve high levels of transfection efficiency, however, the creation of viral vectors and production of viral stocks require a high level of user knowledge, are labor and time intensive, have limited cell type flexibility, and can impact yield due to purification challenges. MaxCyte's proprietary flow electroporation technology is a universal transient transfection platform that provides a practical solution to the time, labor, and cost challenges of stable cell lines while overcoming the limitations associated with other transient transfection methods.

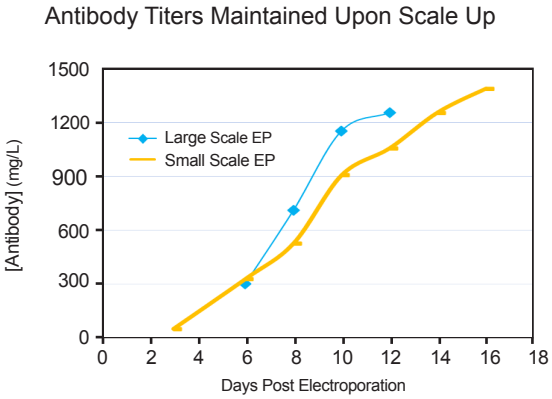
MaxCyte Transient Transfection

MaxCyte flow electroporation combines superior performance, broad applicability, and ease of use with the capacity to transfect up to 2E11 cells in under 30 minutes, creating a fully scalable transfection method. This technology rapidly (co)transfects a wide range of cells including CHO, HEK, CAP-T®, Vero, insect cells, and other cell lines commonly used for protein expression (Figure 1). MaxCyte transfection does not require specialized constructs, engineered cells, media additives, or chemical reagents and consistently results in high levels of transfection efficiency and cell viability. This enables high-yield production of gram-scale quantities of proteins such as mono- and multi-specific antibodies, VLP's, antigens, and other recombinant proteins within days of transfection. This single transfection technology takes you from the production of milligrams of protein for early-stage development through production of multiple grams of protein and generation of stable cell pools and high-yield stable cell lines further streamlining scale-up to biomanufacturing.

MaxCyte STX and VLX Transfection Systems

MaxCyte offers two benchtop, flow electroporation systems, the MaxCyte STX® Scalable Transfection System and the MaxCyte VLX® Large Scale Transfection System that fit the needs of large-scale transient gene expression for protein production. The MaxCyte STX® has the flexibility to transfect from 5E5 cells up to 2E10 cells, while the MaxCyte VLX® is ideal for extremely large-scale transfections with the capacity to transiently transfect up to 2E11 cells. The large capacity and high performance of both these systems easily support the large-scale production of proteins.

All MaxCyte systems are supplied with a pre-loaded library of electroporation protocols optimized for a wide variety of cell types including cells commonly used for protein production such as CHO, HEK, NS0, Vero, and insect cells. These simple push-button systems result in highly reproducible, quality transfections. Additionally, MaxCyte systems are ISO certified and GMP compliant



	STX Small Scale (20mL culture)		STX Large Scale (2.8L culture)		VLX Capacity* (50L culture)	
Days post EP	IgG Titer	Total IgG Produced	IgG Titer	Total IgG Produced	IgG Titer*	Total IgG* Produced
Day 8	575 mg/L	12 mg	700 mg/L	1.96 g	638 mg/L	31.9 g
Day 12	1.08 g/L	**22 mg**	1.22 g/L	**3.42 g**	1.1 g/L	**55.0 g**

Figure 2: High Titer Antibody Production from CHO Cells. *CHO-S cells were transfected with an antibody expression plasmid (2 µg DNA/1E6 cells) via small-scale (8E7 cells) or large-scale (1E10 cells) MaxCyte STX electroporation. Cells were seeded at 6E6 cells/mL post electroporation. 1 mM 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. Secreted IgG titers were measured via ELISA on various days post transfection, and total IgG production calculated. *Results for a VLX transfection were projected based on large- and small-scale average titers observed during the STX study.*

with a Master File on record with the USFDA and Health Canada.

Case Study 1: Quality CHO Cell Antibody Production

While stable CHO cell lines remain the regulatory standard for manufacturing of clinical-grade biotherapeutics, industry has looked to CHO TGE to reduce reliance on stable cell line generation for early- and mid-stage development activities. Initial CHO-based TGE activities were limited by poor transfection efficiencies, cell viabilities, and production of insufficient quantities of antibodies. Many CHO transient transfection methods, including various PEI and lipid-based reagents and protocols, report antibody titers ranging from 2 to 250 mg/L upon full optimization (2-6) limiting their usage for large-scale antibody production. MaxCyte electroporation enables high efficiency, high viability CHO cell transfection routinely resulting in secreted antibody titers >400 mg/L, which can exceed >1 g/L with optimization of post transfection culture conditions (Table 1, Figure 2). This translates into production of multi-gram quantities of antibodies within weeks of a single transient transfection.

Rapid, Scalable Protein Production

Protein production using stable cell lines or viral delivery methods is time- and resource-intensive and can take

months to implement. While transient transfection is a rapid means of protein expression, not all transient systems have the scalability to simply and rapidly produce the full range of protein quantities needed throughout biotherapeutic and vaccine development stages. Many alternative transfection technologies require multiple small-scale transfections, re-optimization of transfection protocols, and/or bulk usage of costly transfection agents for production scale-up. MaxCyte flow electroporation has unmatched scalability, able to transfect as few as 5E5 cells up to as many as 2E11 cells without protocol re-optimization, allowing the progression from gene to gram-scale quantities of proteins within two weeks.

Comparison studies of small- and large-scale CHO electroporation produced similar secreted antibody titers, illustrating the ability to scale-up transfection without re-optimization (Figure 2). Antibody titers of greater than 1.2 g/L were detected using post transfection culture conditions optimized for antibody productivity. Approximately 3 grams of antibody was produced 12 days post transfection from a CHO culture of less than 3 Liters. Scale-up to the MaxCyte VLX allows for transfection of up to 2E11 cells, which would equate to production of over 50 grams of antibody within two weeks of transfection using a single transient transfection.

Highly Reproducible

CHO-based IgG production studies confirm the consistency of electroporation scale-up as well as the reproducible performance of MaxCyte technology. Ten CHO cell electroporations (7 small scale and 3 large scale) were conducted on four different days. High-level transfection efficiencies and cell viabilities routinely produced secreted antibody titers of 400 mg/L when using standard, unoptimized post transfection culture conditions (Table 1). Comparable antibody titers were observed during production studies not only for small- versus large-scale electroporations, but also from day-to-day runs.

Consistent, High Yield CHO Antibody Production

Date	Transfection Scale	Titer (mg/L)	Specific productivity (pg/c/d)
March 20, 2012	Large Scale	396	7.4
March 20, 2012	Small Scale	351	10.4
April 24, 2012	Large Scale	328	6.5
April 24, 2012	Small Scale	337	8.3
April 24, 2012	Small Scale	464	7.3
April 24, 2012	Small Scale	334	12.3
June 12, 2012	Large Scale	453	4.0
June 12, 2012	Small Scale	459	3.9
July 3, 2012	Small Scale	517	11.5
July 3, 2012	Small Scale	455	15.6
Large Scale	**Avg. ± stdv**	**392 ± 43**	**6.0 ± 1.3**
Small Scale	**Avg. ± stdv**	**416 ± 65**	**9.9 ± 2.9**
Total	**Avg. ± stdv**	**409 ± 61**	**8.7 ± 3.3**

Table 1: Consistent and Reproducible Production of Antibodies Using Small- and Large-Scale MaxCyte Electroporation of CHO Cells. *CHO-S cells were transfected with an antibody expression plasmid (1 µg DNA per 1E6 cells) via small-scale (static) or large-scale (flow) electroporation using the MaxCyte STX. Ten electroporations were conducted over the course of four different days. Post electroporation, cells were inoculated at a viable cell density of 4.7E6 ± 0.9 cells/mL. Secreted antibody titers were measured via ELISA 2 weeks post transfection.*

Superior Production of High Quality Antibody-like Molecule

Transfection Method	Purified Protein Concentration	%HMW	% Monomer
STX Electroporation	**173 mg/L**	5.6	94.3
Lipid-based Reagent	7.3 mg/L	7.2	92.8

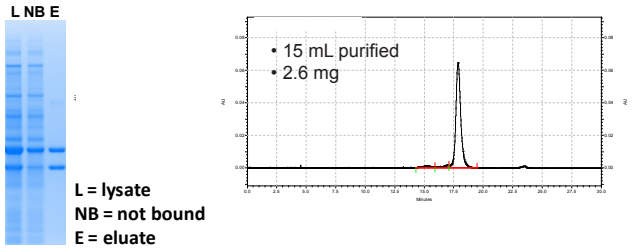


Figure 3: Production of Quality Bispecific Antibodies. *CHO-S cells were transfected with a bicistronic expression plasmid encoding the components of a bispecific diabody via static electroporation in an OC-400 processing assembly or using a standard lipid-based reagent protocol. Diabodies were purified and subjected to analysis via chromatography.*

Case Study 2: Antibody-like Protein Production

A diversity of antibody-like molecules including a host of bi-specific antibody types, tri-functional antibodies, various antibody fragments (Fabs, scFv, hetero-dimerization domains), and IgG-fusions have been bioengineered in hopes of improving the therapeutic potential of standard antibodies. The efficacy of many of these formats has been demonstrated in preclinical and clinical studies and, thus, antibody-like molecules are of particular interest for continued development (7). These molecules can, however, be difficult to express at higher yields from mammalian cells. The proven high cell viability and transfection efficiency of MaxCyte electroporation enable production of a variety of antibody-like proteins and peptides.

Production of Quality Bispecific Antibodies

MaxCyte electroporation was compared to a lipid-based reagent system for expression of a diabody via CHO cell transient transfection (Figure 3). Diabody titers were more than twenty fold higher using MaxCyte electroporation. Analysis of the purified diabodies showed that greater than 94% of the MaxCyte produced protein was in the desired monomeric form. A single MaxCyte electroporation produced a total of 2.6 mg of purified diabody from a 15-mL culture equating to production of 1.7 grams of diabody from a 10-L culture.

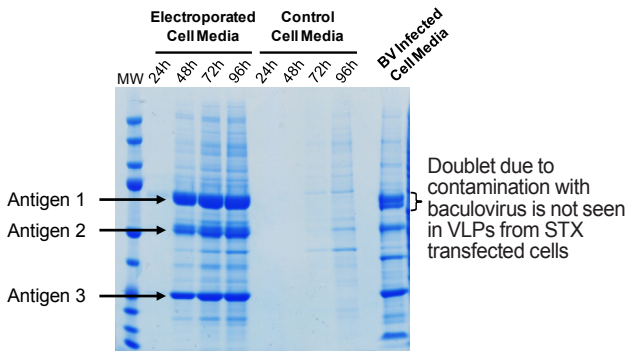


Figure 4: Sf9 VLP Production Using MaxCyte Electroporation: Plasmid to Protein in Two to Four Days. *Sf9 cells were transfected via small-scale electroporation with a single plasmid encoding three antigens that co-assemble into VLPs. Culture media was collected at various times from cells post EP or following baculovirus infection and analyzed using SDS-PAGE.*

Case Study 3: Insect Cell VLP Production

Insect cells offer an attractive alternative to mammalian cells for biomanufacturing as they post-translationally modify proteins in a manner similar to that of mammalian cells yet have many advantageous features related to ease of culturing such as simplified cell growth readily adapted to high-density suspension. While both transient transfection and recombinant baculovirus platforms are commonly used methods for insect cell protein expression, high efficiency transient transfection offers a more rapid and straightforward means of protein production.

Despite development of specialized media, transfection reagents, and baculovirus vectors aimed at simplifying gene cloning and reducing overall implementation timelines, baculovirus-mediated protein production remains an extended, multi-stage process. This 6- to 8-week process requires construction of expression plasmid(s), transfection of insect cells, viral stock preparation, and subsequent infection of insect cells all prior to final production and purification of the recombinant protein of interest. In contrast, MaxCyte electroporation directly transfects Sf9, Sf21, and SL3 cells with >90% cell viability and transfection efficiency levels, allowing for rapid, high-level protein expression (Figure 1 and data not shown).

VLP Production Using Transient Transfection of Sf9 Cells

VLPs are complex, multiprotein structures of self-assembling viral antigens that are a promising new type of vaccine and commonly produced in insect cells. Sf9 cells transfected via MaxCyte electroporation with an expression construct encoding three antigens that co-assemble into a VLP resulted in significant secretion of VLPs within 48 hours post transfection (Figure 4). In tandem, a baculovirus expression system was used to produce VLPs containing the identical three antigens. Cell supernatants were collected from baculovirus-infected Sf9 cells and Sf9 cells directly transfected with the expression construct. SDS-PAGE analysis shows the presence of the three VLP antigens in all electroporation and baculovirus samples; however, baculovirus protein contaminants were also present in supernatants from baculovirus-infected cells. This is consistent with the literature (8, 9), which documents the propensity for baculovirus protein contamination when using these expression systems. Overall, these results demonstrate the extremely rapid and high quality nature of direct insect cell transfection using MaxCyte electroporation, which can further streamline protein production by eliminating the need for baculovirus usage even for more complex molecules such as VLPs.

Conclusions

Flow electroporation is a proven means of rapid, high-performance transient (co)transfection of mammalian and insect cells supporting large-scale production of antibodies, antibody-like molecules, recombinant antigens, VLPs, and vaccines. High transfection efficiencies and cell viabilities enable production of gram to multi-gram quantities of proteins within days of a single transfection. Additionally, flow electroporation can be used to generate stable pools and cell lines further increasing its utility and streamlining of development pipelines. The MaxCyte STX® and MaxCyte VLX® have unmatched quality, flexibility, reproducibility, and scalability, creating a single, cost-effective platform that supports the full range of biotherapeutic and vaccine development activities from candidate identification and development through biomanufacturing.

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