Using Flow Electroporation to Find More Relevant Candidates and Shorten the Antibody Development Timeline

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Abstract

As the number of biotherapeutics grows, success in the coming years will depend on the ability to get to market quickly. This will involve the ability to work upstream in the manufacturing cell line. While transient gene expression is a rapid and cost-effective means of protein production, particularly during early development and preclinical stages, most transient transfection methods do not meet the requirements of scalability, consistency, speed, and cell type flexibility. MaxCyte flow electroporation enables rapid transient transfection, producing high cell viabilities and transfection efficiencies for a variety of commonly used cell lines in manufacturing. In this poster, data will be presented that shows the ability of the MaxCyte STX® Transfection System to transfect CHO cells with high efficiency and viability, enabling R&D to be conducted in the manufacturing cell line. A comparison of different transfection methods will be presented in CHO and HEK cells.

MaxCyte Transient Transfection Platform

MaxCyte STX®
5E5 Cells in Seconds
Up to 2E10 Cells in <30 Min

MaxCyte VLX®
Up to 2E11 Cells in <30 Min

The MaxCyte STX® and MaxCyte VLX® Transient Transfection Systems use fully scalable flow electroporation for rapid, highly efficient transfection.

- High efficiency & high cell viability
- Broad cell compatibility
- Streamlined scalability requiring no re-optimization
- Single use processing assemblies for simplified bioproduction

Choosing A Host Cell

Glycosylation Patterns of Common Host Cells

Use the Most Relevant Host Cell to Get the Most Relevant Candidate

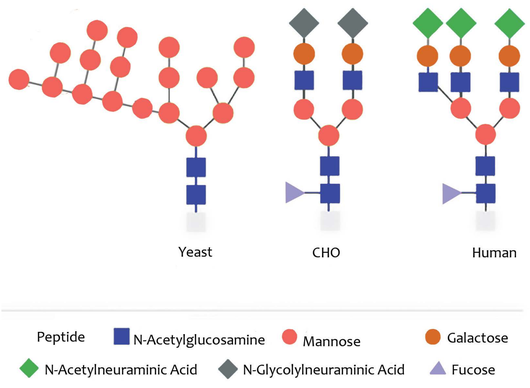
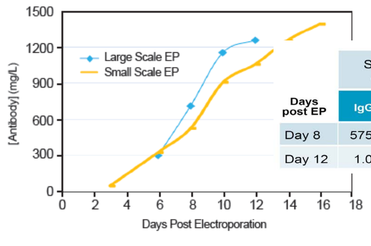


Figure 1. Different Expression Hosts have Different Glycosylation Patterns. For more information, see **1**. Croset, A. etc. (2012) Differences in the glycosylation of recombinant proteins expressed in HEK and CHO cells. *J. Biotech.***2.** Zeck, A. etc. (2011) Cell type-specific and site directed N-glycosylation pattern of FcγRIIIa. *J. Proteome Res.* ***3***. Bulter M and Spearman, (2014) The choice of mammalian cell host and possibilities for glycosylation engineering . *Current Opinion in Biotech.*

Gram-Scale CHO Antibody Production

>3 grams of Antibody from <3-Liter Culture



Days post EP	STX Small Scale (20mL culture) IgG Titer	STX Small Scale (20mL culture) Total IgG Produced	STX Large Scale (2.8L culture) IgG Titer	STX Large Scale (2.8L culture) Total IgG Produced	VLX Capacity* (50L culture) IgG Titer*	VLX Capacity* (50L culture) 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 Maintained Upon Scale-Up. 8E7 or 1E10 CHO-S cells were transfected with an antibody expression plasmid (1 µg DNA/1E6 cells) via small-scale (static) or large-scale (flow). 1 mM sodium butyrate was added to cultures and the temperature lowered to 32°C 24 hours post EP. Cultures were fed daily with an optimized media. Secreted IgG titers were measured via ELISA on various days post EP, and total IgG production calculated. 1E10 CHO cells transfected yielded **>3 g of antibody from a 2.8-L culture**. *Results for a MaxCyte VLX transfection (2E11 cells) were projected based on the average titer for large- and small-scale transfections during the STX study. These data suggest that **>50 g of antibody** could be produced following a single, 30-minute VLX electroporation.

Highly Efficient DNA Loading in CHO Cells

>95% Viability and Transfection Efficiency

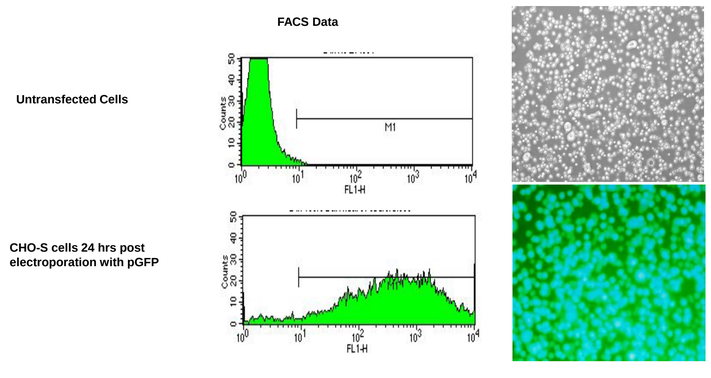


Figure 3. Greater then 95% CHO Cell Transfection Efficiency and Cell Viability Using MaxCyte Transient Transfection. CHO-S cells were transfected with a plasmid encoding green fluorescent protein (2 µg DNA/1E6 cells) using small-scale (static) electroporation on the MaxCyte STX. GRP expression and viability were measured by flow cytometry (FACS) 24 hours post electroporation.

Superior, Consistent Transient Transfection

Reliable, Fast Antibody Expression

MaxCyte Platform Outperform Other Methods

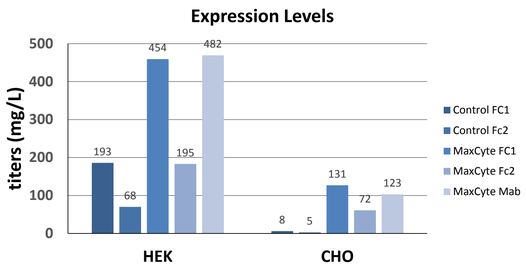


Figure 4. Transient Expression of Ig-based Chimeric Fusion Proteins in HEK and CHO Cells. Suspension-adapted HEK and CHO cells were transfected with plasmids encoding two different Ig-based chimeric fusion proteins. Control samples were transfected with a chemical transfection reagent; MaxCyte samples were transfected via static EP in OC-400 processing assemblies. Total secreted protein concentrations were measured after 5-7 days of culture in 125-mL shake flasks. MaxCyte titers were 2.5 fold higher in HEK cells and 20 fold higher in CHO cells relative to chemical-based transfection.

Production of Quality Antibodies

MaxCyte Transfection Provides Dependable Quality

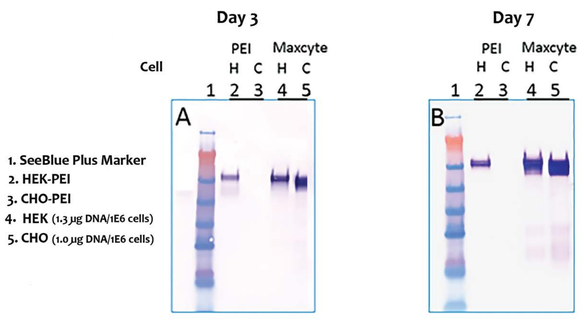


Figure 5. SDS-PAGE Analysis of His-tagged Proteins Transiently Expressed in HEK and CHO Cells. Suspension-adapted HEK and CHO cells were transfected with plasmids encoding a His-tagged fusion protein using PEI or static electroporation with the MaxCyte STX. Equal volumes of conditioned media were removed from each culture on days 3 and 7 post transfection. Total proteins were separated via electrophoresis on a 4-12% SDS polyacrylamide gel and subjected to western blotting with an anti-His tag antibody. The results indicate substantially higher titers, particularly in CHO cells, using the MaxCyte STX. Protein integrity is confirmed by the absence of lower molecular weight bands.

High Titer Bispecific Antibodies

MaxCyte Transfection: High Titer and Quality

Expression (purified protein)	%HMW	%Monomer
173 mg/L	5.6	94.3

STX-produced diabody:
Protein A capture &
chromatography data

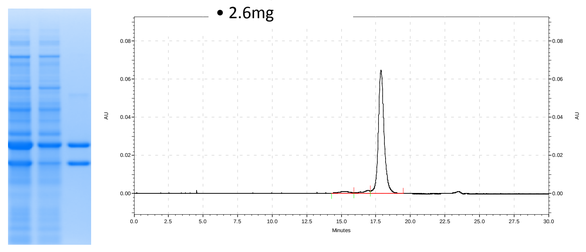


Figure 6. Production of Quality Bispecific Antibodies. CHO-S cells were transfected via static electroporation in an OC-400 processing assembly with a bicistronic expression plasmid encoding the components of a bispecific diabody. Total secreted diabody concentrations were measured using ELISA on various days post transfection. Diabody titers were more than 20-fold higher using MaxCyte electroporation. Analysis of purified proteins showed that most of the MaxCyte-produced protein was in a monomeric form.

Reproducible, Scalable Transient Transfection

Day-to-Day & Batch-to-Batch Consistency

Date	Transfection Scale	Titer (mg/L)
March 20	Large Scale	396
March 20	Small Scale	351
April 24	Large Scale	328
April 24	Small Scale	337
April 24	Small Scale	464
April 24	Small Scale	334
June 12	Large Scale	453
June 12	Small Scale	459
July 3	Small Scale	517
July 3	Small Scale	455
Total	**Avg. ± stdv**	**409 ± 61**

Table 1. Consistent, 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/1E6 cells) via small-scale (static) or large-scale (flow) electroporation. Ten electroporations were conducted over 4 days. Post electroporation, cells were inoculated at a viable cell density of 4.7E6 ± 0.9. Secreted antibody titers were measured via ELISA 2 weeks post transfection.

Summary

- MaxCyte transfection scalability allows for its seamless use in both scaled-down process development and optimization studies as well as scaled-up for bioproduction.
- The MaxCyte STX enables rapid optimization of a variety of post transfection conditions including cell density, temperature shift, media composition and additives, and feed schedule.
- MaxCyte EP can produce antibody titers in CHO cells >1.2 g/L using optimized conditions.
- Scale up from small- (static) to large-scale (flow) electroporation does not require re-optimization while maintaining transfection performance. Additionally, scaling from the MaxCyte STX to the MaxCyte VLX results in comparable performance.
- MaxCyte EP is highly reproducible, allowing for consistent batch-to-batch bioproduction.
- MaxCyte EP is a versatile platform for production of simple proteins like antibodies as well as protein complexes such as lentivirus.

Benefits of MaxCyte Flow Electroporation

- MaxCyte electroporation protocols and scale-up from bench to manufacturing are optimized and computer controlled, with reproducible results from day-to-day and operator-to-operator. There is no reoptimization of reagents or upstream/ downstream scale changes, saving time and allowing more campaigns with the same resources.
- The ability to transfect a large number of cells (2E10) allows for the generation of antibodies for screening along with clonal selection from the same cell population, saving time and resources and improving consistency.
- Different cell lines produce antibodies that can differ in structure, potency, and binding. Working directly in the manufacturing host cell in development generates the most relevant data for candidate selection, saving time and resources and accelerating market release.
- MaxCyte electroporation uses cells at a high density, enabling process volume reductions to 20-25% of that required for reagent-based transfection methods. Process and time savings can be realized from reduced media & feed needs, smaller reaction vessels, simplified & shorter filtration/purification cycles, and impacts on other operations.

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