Scalable, High-Yield Protein Production via Flow Electroporation: Expanding the Use of Transient Gene Expression in Biotherapeutic Development.

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Abstract

A variety of factors - including time, resources, quantity requirements, price, as well as scientific drivers such as post translational modifications, protein folding, and regulatory implications - drive the method chosen for protein production during biotherapeutic development. Transient gene expression (TGE) is frequently used during early-stage development to rapidly and cost-effectively produce milligram quantities of proteins, followed by migration to stable cell line generation for mid-stage development through biomanufacturing. MaxCyte's unique transient transfection technology meets the scalability, consistency, and cell type flexibility needed for broad application of TGE in biotherapeutic development pipelines. MaxCyte's flow electroporation-based TGE can be used for the production of antibodies, antibody-like molecules, and recombinant proteins from a variety of cell types such as CHO, HEK, CAP-T, and insect cells. In this poster, data will be presented on the scalable production of CHO antibody titers >1 g/L. The ability to producing multi-gram quantities of proteins with MaxCyte's electroporation TGE for early- and mid-stage development delays migration into stable cell lines, streamlining the biotherapeutic development process.

MaxCyte Transient Transfection Systems

MaxCyte STX®

5E5 Cells in Seconds
Up to 1E10 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 with very high cell viability post transfection. Transfected cells support gram scale production of antibodies and proteins for efficient biotherapeutic development.

- Rapid & simple to use
- High efficiency & high cell viability
- CHO, HEK, NS0, Vero & Insect cell compatibility
- Streamlined scalability requiring no re-optimization

Cell Type Flexibility for Protein Production

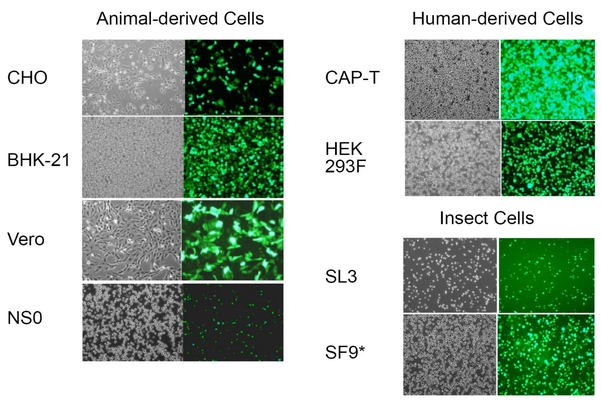


Figure 1. High Efficiency Transfection of Cell Types Commonly Used for Protein Production. Various cells were transfected with 2 µg/1E6cells of pGFP DNA using the appropriate MaxCyte STX protocol. Cells were examined for GFP expression using fluorescence microscopy 24 hrs post electroporation.

Superior Transient Transfection

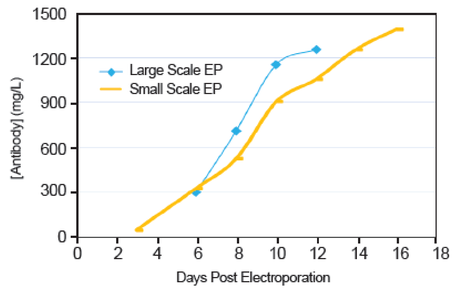
Cell Type	Protein Type	Previous Transfection Method	Previous Method Titer (mg/L)	MaxCyte Titer (mg/L)	Fold Increase
CHO	IgG	PEI	30	210	7
CHO	IgG	PEI	<1	10.3	10
CHO	IgG	PEI	21	227	10.8
CHO	Viral Protein	PEI	4.5	23.1	5.1
CHO	Bi-specific Ab	Lipid	7.3	173	23.7
CHO	IgG	Lipid	29	227	7.8
HEK	IgG	Lipid	51	250	4.9
HEK	IgG	Lipid	12.5	87.7	7.0
CAP-T™	IgG	PEI	62.5	146.5	2.3
SL3	Secreted Protein	PEI	0.8	52	65

Table 1. Routinely Higher Protein Production Following MaxCyte Transfection. These data represent a compilation of side-by-side transfection comparing MaxCyte electroporation to PEI or lipid-based reagents. The type of cell and general description of the expressed protein are noted. MaxCyte electroporation routinely outperforms other chemical and lipid-based transfection methods with >2 to 65-fold increases in protein titers.

Consistent, Scalable High Titer Antibody Production

Gram Scale CHO Antibody Production

>1.2gram/L Antibody Titers With Optimized Feed



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

Figure 2. High Titer Antibody Production Maintained Upon Scale Up. 8E7 or 1E10 CHO-S cells were transfected with an antibody expression plasmid (1µg DNA/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 secreted IgG concentrations were measured using ELISA on various days post transfection. 1E10 CHO cells transfected using a single electroporation run yielded **>3g of antibody from a 2.8L culture**. The MaxCyte VLX has the capacity to transfect 2E11 cells which would equate to the production of over 50 grams of antibody from a single 30 minute transient transfection.

Reproducible, Scalable Transient Transfection

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

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 2. 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 (1µg 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. Secreted antibody titers were measured via ELISA 2 weeks post transfection.

Bioproduction Scale Up

Fully Scalable Transient Production

Seamless MaxCyte STX to VLX Transition

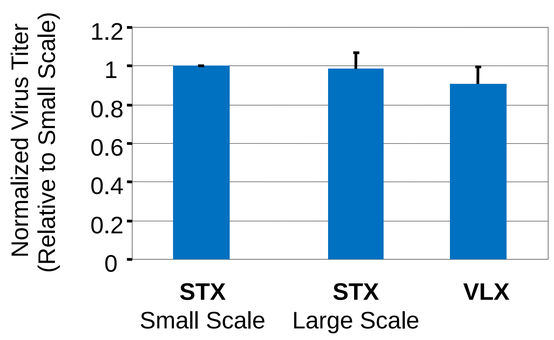


Figure 3. Scale Up of Lentiviral Vector Production from Small-Scale to Large-Scale Production Using the MaxCyte Platform. Suspension-adapted HEK 293FT cells were suspended in MaxCyte's electroporation (EP) buffer at 1E8 cells/mL. A mixture of plasmids encoding lentiviral vector components was added to the cells (0.4µg of DNA/1E6 cells), and cells were transferred to sterile OC-400, CL-2 and VLXD processing assemblies. Cells in the OC-400 and CL-2 were transfected by static and flow EP, respectively, using the STX instrument; cells in the VLXD were transfected by flow EP on the VLX. Lentiviral titers were measured after 24-48 hrs in culture. Normalized titer data show seamless scalability of the MaxCyte transfection process.

Stable Cell Generation

Rapid Generation of Stable Clones

Antibody Titer >3g/L in 6 Weeks

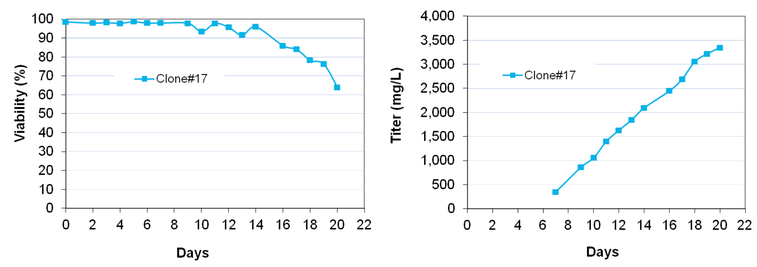


Figure 4. Rapid Stable Cell Line Development Using MaxCyte Flow Electroporation. Humanized mAb DNA was transfected into CHO-S cells using STX technology. Limiting dilution cloning was carried out from the stable pool in selection media. Cell lines were generated at week 6 and scaled up for the production and accession cell bank. Productivity of the selected clone, achieved in less than 8 weeks from start to finish, was >3g/L as a fed batch.

Summary

- MaxCyte electroporation of CHO, HEK, CAP-T™, and other cell types commonly used in biotherapeutic development results in high cell viability and high titer antibody production.
- MaxCyte transient transfection routinely leads to significantly higher levels of production for a variety of protein and cell types.
- Optimization of conditions such as post transfection cell media and feed strategy allow for extremely high antibody titers which can **exceed 1gram/L**, enabling production of over **3.4 grams** of antibody from a single transfection of 1E10 cells.
- MaxCyte transfection produces consistent batch-to-batch and day-to-day results for both small (static), and large scale (flow) electroporation.
- The MaxCyte platform allows for streamlined TGE scale up from 5E5 cells up to 2E11 cells for the potential production of 50 grams of antibody from a single transfection.
- High yield stable cell lines can be rapidly developed in 6-8 weeks without using specifically engineered vectors or CHO cell lines, due to high transfection efficiency and cell viability.

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