Rapid Transient and Stable Protein Production with Consistent Quality to Accelerate Biotherapeutic Development.

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Abstract

Rapid recombinant protein production is one of the key elements for drug development success. However, to consistently produce good quality biotherapeutic molecules transiently is still quite challenging due to consistency and scalability issues. Therefore, high levels of process consistency and scalability are important not only for GMP stage manufacturing, but they are also critical for early stage R&D studies since good predictivity of any scale production can shorten timelines and minimize costs. The aim of this study is to show how Flow Electroporation™ Technology can transiently produce therapeutic materials from milligram to gram scales quickly to support early to mid-stage drug development. We will present our data to demonstrate a) the robustness of the platform for protein expression in different cell lines (Antibody titers of 2.7 g/L were obtained by transient gene expression in CHO-S cells), b) the great scalability and consistency of the technology from 0.5 x 106 to 2 x 1011 of the transfected cells and c) the comparable protein quality between transient expression lots and also comparable quality to protein produced by a stable cell line. So the high transient productivity, product quality and scalability in CHO cells by using Flow Electroporation™ Technology can accelerate the drug development process and reduce the risk of drug evaluation and selection. And potentially transient expressed recombinant proteins could be used for clinic study in future.

Universal, High-Level Transfection Efficiency and Cell Viability

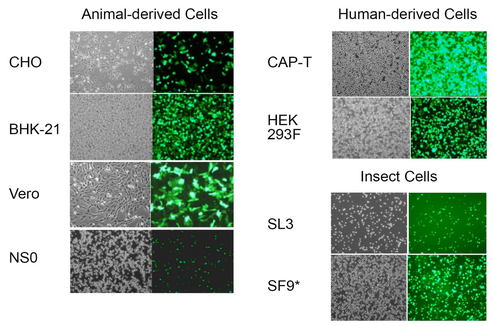


Figure 1. High Efficiency Transfection of Cell Types Commonly Used for Protein and Vaccine Production. Various cells were transfected with 2 µg/1E6 cells of pGFP DNA using the appropriate MaxCyte STX protocol. Cells were examined for GFP expression using fluorescence microscopy 24 hrs post electroporation.

Multi-Gram, CHO-based Antibody Production

>500 mg/L Achieved in <1 weeks OR > 2.7 g/L Antibody Titers in <3 Weeks

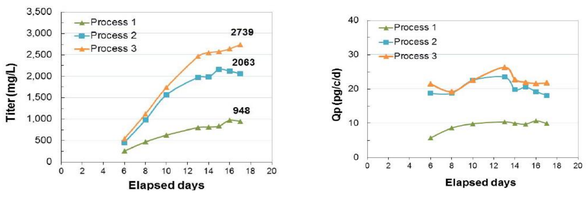
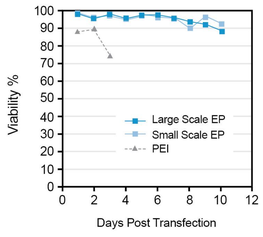


Figure 2. Post Electroporation Culture Process Optimization Produces CHO-S Antibody Titers >2.7 g/L. CHO-S cells were transfected with an antibody expression plasmid (1µg DNA/1E6 cells) via small scale electroporation on the MaxCyte STX. Cells were plated at approximately 4E6 cells/mL post electroporation. Transfected cells were cultured in media with different additives and culture conditions. Titer was verified by both ELISA and Protein A capture assays. The optimized process produced antibody titers of 2.74 g/L at day 17 post EP as a fed batch.

Rapid Production of Recombinant Proteins for Use as Vaccines

High Cell Viability Leads to Strong HIV gp145 Expression

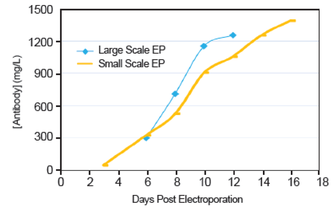


Day Post Transfection	gp145 (mg/L)		
	MaxCyte		PEI
	Small-scale	Large-scale	
3	27.8	23.1	4.5
7	67.2	75.1	-
10	95.2	113.5	-

Figure 3. Cell Viability and Protein Titer Data Following Transfection of CHO Cells with an HIV Envelope Protein Expression Plasmid. CHO-S cells were transfected with a gp145 expression plasmid via small scale EP (8e7 cells) and large scale EP (2e9 cells). Transfected cells were inoculated into shake flasks at the same density and cultured for 10 days. Cells from the small scale and large scale EPs yielded consistent titers and exhibited high viabilities. Viabilities and titers from the electroporated cells were much higher than corresponding values for a customer's optimized PEI process.

Scalable, Gram-Level CHO Antibody Production

Simple Transfection Scale Up



	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 4. High Titer Antibody Production Maintained Upon Scale Up. CHO-S cells were transfected with an hIgG plasmid (1µg DNA/1E6 cells) via small- or large-scale 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.

True Scalability - MaxCyte STX to MaxCyte VLX Scale Up

Viability, Growth & Antibody Titers Maintained with No Reoptimization

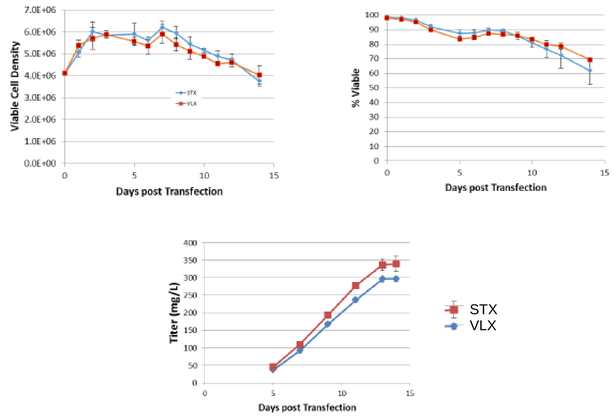


Figure 5. MaxCyte STX to VLX Instrument Scale-up Exhibits Consistent Protein Titers, Cell Viabilities, and Growth Kinetics. Duplicate sets of 2E10 CHO-S cells were transfected with an hIgG1 expression plasmid (1ug/1E6 cells) via flow electroporation with the MaxCyte STX or the VLX. Transfected cells were seeded into 1 L cultures at a starting density of 4E6 cells/mL, and fed batch cultures were maintained for 15 days. Secreted protein titers were monitored by ELISA; cells counts and viabilities were measured via trypan blue staining. Error bars denote standard deviations from three replication shake flasks.

Rapid, High-Yield Stable Cell Generation

Stable Cell Lines Generated in 6 Weeks

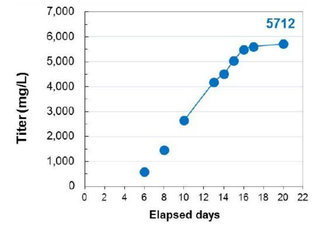
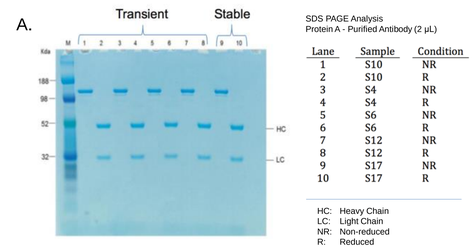


Figure 6. Rapid Identification of High-Yield Stable Clone. A stable pool of CHO cells expressing an hIgG was generated within two weeks of electroporation. 479 clones were screened following limited dilution cloning. The top clone was selected for production within 6 weeks post transfection. Production was carried out in shake flasks as a fed batch. At day 17, productivity reached 5.5 g/L. Results were verified by both ELISA and Protein A capture assays.

Equivalent Antibody Quality & Glycosylation

Similar IgGs Produced via MaxCyte Transient Transfection & Stable Cell Lines Generated Using MaxCyte Transfection



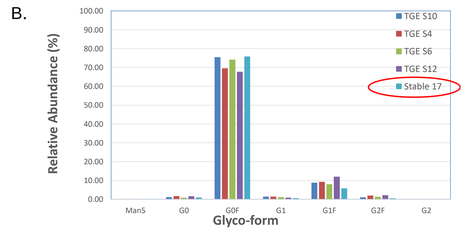


Figure 7. hIgG1 Quality & Glyco-form Comparison - Transient vs Stable Expression. A hIgG was expressed transiently in CHO-S cells via four independent small-scale electroporations with the MaxCyte STX. A stable cell line (S17) was also generated by subjecting transfected cells to antibiotic selection, followed by limited dilution cloning. A). SDS-PAGE gel analysis (reducing and non-reducing) data indicate equivalent quality (i.e aggregation or degradation) of antibodies produced via transient or stable transfection. B.) Glycoform analysis showed highly consistent patterns of post-translational modification among the different transient transfection runs as well as the protein produced from media harvested from the stable cell line.

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
- True scalability requiring no re-optimization
- Closed, computer-controlled instruments
- cGMP-compliant & CE-marked
- Master file with US FDA & Health Canada

Summary

- The high transfection efficiency and cell viability rates of MaxCyte's electroporation-based, delivery platform enable rapid production of therapeutics molecules via transient or stable expression leading to accelerated candidate development.
- Flow Electroporation™ Technology is universal in nature and can produce a variety of high quality, functional protein types including full IgGs, bispecifics, tribodies and Fc fusion proteins (data not shown).
- Transient transfection of CHO cells produces the gram-scale quantities of antibodies needed for early and mid-stage development reducing early reliance on stable cell generation.
- Antibodies (IgG) transiently produced via Flow Electroporation™ Technology exhibit glycosylation and protein characteristics similar to stably produced protein, supporting the use of transiently produced proteins in early-stage discovery effects.
- High CHO cell viability post electroporation enables rapid generation of stable pools and development of stable clones in 6 weeks with yields >5.7 g/L. High cell viability enabled stronger antibiotic selection which enriches for high yield clones. Fewer than 500 clones needed to be screened to identify a high-yield stable cell line.
- The MaxCyte Scalable Transfection Systems offer a highly consistent delivery platform that provides the speed, scalability and cGMP compliance for use in biomanufacturing of therapeutics and vaccines.

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