Scalable Antibody Production from CHO Cell Line of Choice Using Flow Electroporation.

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Abstract

One aspect of lowering costs and reducing attrition rates during biotherapeutic development is the ability to work in the cell line of choice during early-stage discovery. The MaxCyte transfection platform offers a universal means of high efficiency, cell type flexible, and fully scalable protein expression. From a single CHO transient transfection, MaxCyte flow electroporation is capable of producing multiple grams of antibodies. In this poster, data are presented demonstrating the ability of MaxCyte electroporation to produce higher antibody titers in a variety of CHO cell lines compared with alternative transfection methods. The production of CHO antibody titers >2.7 g/L and seamless scalability of MaxCyte electroporation are demonstrated. Lastly, data are shown for the analysis of CHO-based protein quality and glycosylation patterns following MaxCyte transient transfection.

Flow Electroporation: High Performance, Fully Scalable Transient Transfection

CHO-S: Multi-Gram Antibody Production

2.7 g/L Achieved in <3 Weeks

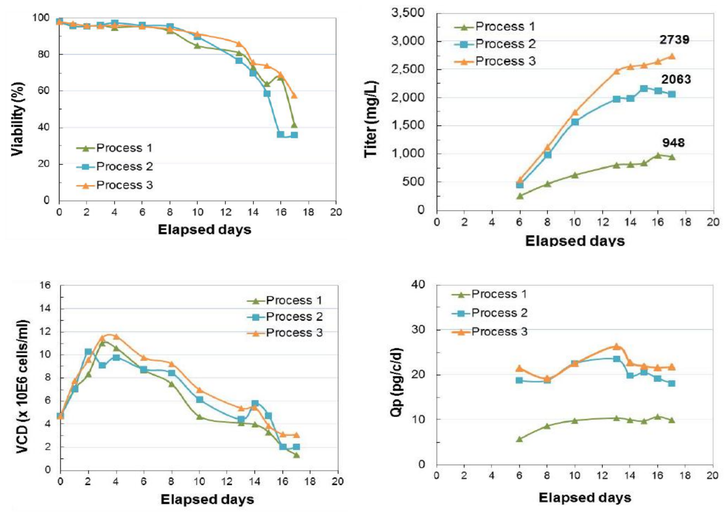


Figure 1: Transient Expression of hIgG1 antibody in MaxCyte EP Transfected CHO-S cells. The same transfected cells were in different production processes. Further optimized process (process3) can reach 2.7g/L as a fed batch. Titer was verified by both ELISA and Protein A capture assays.

Transient Transfection for Bioproduction

Seamless Electroporation Scale-up From MaxCyte STX to VLX

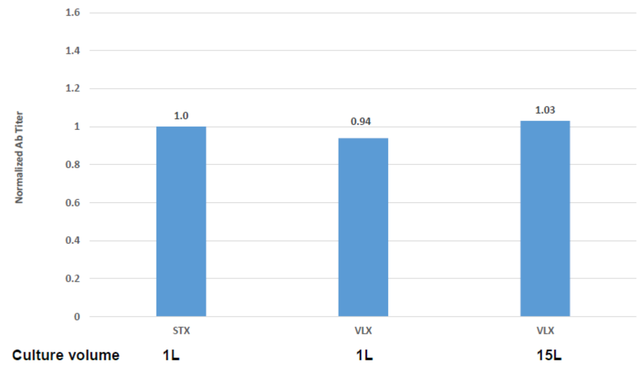


Figure 2: Scale up of CHO-S Cells from Small to Large Scale Using the MaxCyte Platform. Two sets of 2E10 CHO-S cells were transfected by flow EP with an hIgG expression plasmid using the MaxCyte STX and VLX instruments. Following EP, cells were seeded into 1-L shake flasks. Another transfection was performed on the VLX with 2E11 cells, and cells seeded into a 15-L WAVE bag at the same density as cells in shake flasks. Relative titers far all three sets of cells measured two weeks post EP demonstrate reproducibility and scalability of MaxCyte electroporation.

High Performance Transient Transfection of a Variety of CHO Cell Lines

CHO EBNA & 293 EBNA: PEI versus MaxCyte EP

Superior Antibody Expression Using the MaxCyte STX

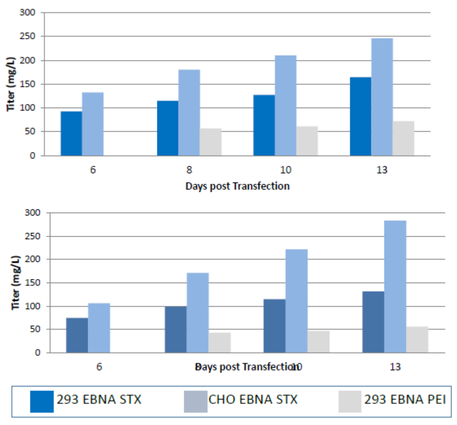


Figure 3: High Titer mAb Expression in CHO EBNA and 293 EBNA Cells. CHO EBNA and 293 EBNA cells were transfected with an IgG expression plasmid via static electroporation (6E7-8E7 cell per condition) and cultured in 125 mL shake flasks for 13 days. Secreted antibody titers in both STX-transfected cell lines greatly exceeded titers generated by an optimized PEI transfection method of 293 EBNA cells.

CHO-K1SV: PEI versus MaxCyte EP

Antibody Titers >1 g/L Within Two Weeks of MaxCyte EP

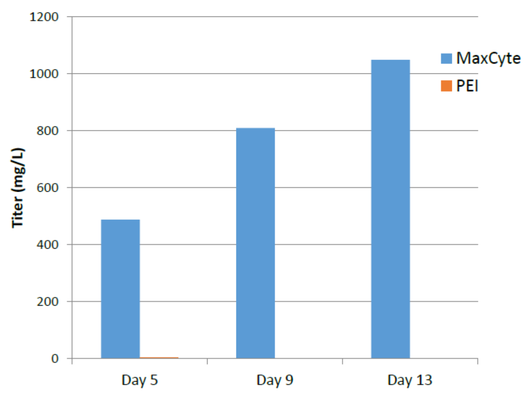


Figure 4: High Titer Antibody Production in CHO-K1SV Cells via MaxCyte EP. CHO-K1SV cells were transfected via static EP or PEI with an IgG expression plasmid and cultured for 13 days or 5 days, respectively. Titer was assayed in the transfected cell on days 5, 9, and 13 post EP. Titers were measured in the PEI transfected cells on day 5. The day 5 titer data indicated clear superiority of MaxCyte EP vs PEI, and the titer data on day 13 revealed productivity exceeding 1 g/L in STX-transfected CHO-K1SV cells.

CHO-K1: MaxCyte STX Outperforms Other Methods

3-24 Fold Higher Protein Titers than Variety of Other Methods

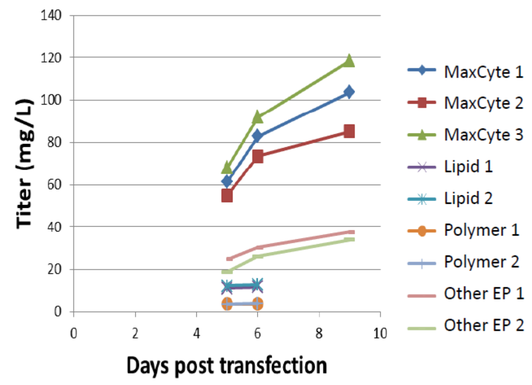


Figure 5: Superior Therapeutic Protein Production in CHO-K1 Cells with the MaxCyte STX Compared to Other Transfection Methods. CHO-K1 cells were transfected via MaxCyte EP, a research-scale electroporation instrument, polymers, or lipid reagents with a plasmid encoding a recombinant protein and cultured in 125 mL shake flasks for up to 10 days post transfection. Titers in three sets of cells transfected with the STX were significantly higher than titers generated by cells transfected via all other methods.

High Quality IgG Production via Transient Transfection of CHOZN® Cells

High Antibody Titers Achieved with MaxCyte

"Out of the Box" Efficient Transient Expression in CHOZN Cells

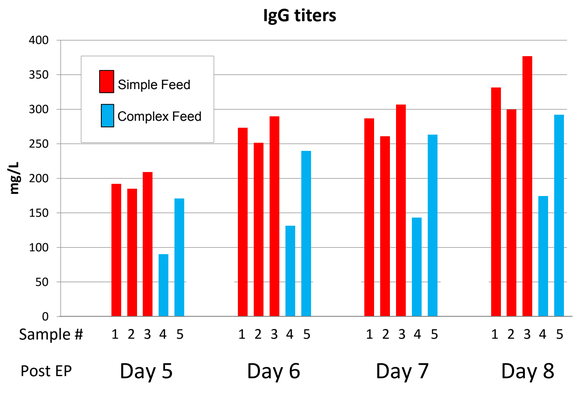
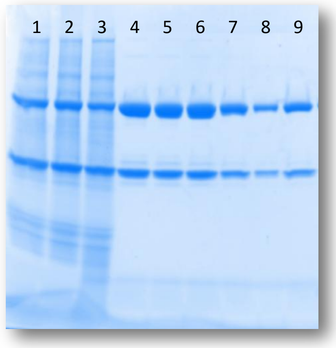


Figure 6: IgG titers in transiently transfected CHOZN® cells. CHOZN® cells were transfected via static electroporation (8E7 cells/condition). Transfected cells were cultured in shake flasks with Ex-Cell® CD Fusion medium (Sigma). Cultures were batch fed with either a simple glucose feed or a more complex mixture of amino acids, sugars, and hydrolysates. Conditioned media samples were collected at Days 5-8 post electroporation (EP), and mAb titers quantified by interferometry using a Protein A probe. CHOZN® cells fed with a simple glucose feed yielded antibody titers exceeding 350 mg/L within 8 days post EP. *All CHOZN data courtesy of Sigma Aldrich.*

Gel Analysis of Protein Quality and Titer

50Kda Heavy Chain and the 25Kda Light Chain Are Clearly Seen



10/10 refers to ratio of 2X sample buffer to protein sample (v/v)

1. sample 1; unpurified 10/10
2. sample 3; unpurified 10/10
3. Sample 5; unpurified 10/10
4. Sample 1 prot. A pure 10/10
5. Sample 3; prot A pure 10/10
6. Sample 5; prot A pure 10/10
7. Sample 1; prot A pure 10/5
8. Sample 3 prot A pure 10/5
9. Sample 5 prot A pure 10/5

Figure 7: SDS PAGE analysis of transiently expressed IgGs. Conditioned media samples from Day 8 post EP were run on a Novex 4-20% SDS PAGE Tris Glycine gel. Gel was stained with Coomassie Blue G-250. Bands of the correct size for hIgG heavy and light chains are clearly evident on a reducing, Coomassie-stained gel loaded with unpurified media samples and Protein A purified samples. No additional bands are evident in the purified samples, indicating good protein quality.

Glycosylation of Stably vs. Transiently Produced IgG

Feeding Protocol Had Greater Impact than Production Method

Reference from Stably Producing Culture

Modifiers	SO57 Ref
Glycosylation G0F	49.0
Glycosylation G1F	38.0
Glycosylation G2F	6.6
Glycosylation G0	5.3
Glycosylation Man5	0.7
Non-glycosylated	0.5

Deconvoluted MW of SO57 heavy chain (G0F), 50395 Da, was within 0.01% of the theoretical MW, 50395 Da, detived with consideration to partial reduction. The reference served to demonstrate system suitability and data were in accordance with historical data.

Transiently Transfected

Simple Feed

Modifiers	7
Glycosylation G0F	43.3
Glycosylation G1F	25.9
Glycosylation Man5	18.8
Glycosylation G2F	6.4
Glycosylation G0	4.6
Non-glycosylated	0.6

Modifiers	9
Glycosylation G0F	43.5
Glycosylation G1F	25.4
Glycosylation Man5	21.2
Glycosylation G2F	5.5
Glycosylation G0	3.5
Non-glycosylated	0.9

Complex Feed

Modifiers	11
Glycosylation G0F	65.7
Glycosylation G1F	23.1
Glycosylation G0	4.7
Glycosylation Man5	3.1
Glycosylation G2F	2.4
Non-glycosylated	0.9

Deconvolution artifacts, common protein modifications and adducts set a limit of detection for this assay at 5-6% of the most intense protein glycoform peak. Give this, care should be taken when considering composition values ≤ 5%.

Figure 8: Glycan analysis of recombinant IgGs. Three sets of transfected CHOZN® cells were cultured in shake flasks with identical base medium. Cells were fed with either a simple glucose feed or a more complex feed solution. Glycan analysis was performed via mass spectrometry on the transiently produced proteins and on a reference protein generated from a stably producing culture in a bioreactor with optimized growth and productivity conditions. Independently transfected cells cultured with the same feed showed consistent patterns of post translational modification.

Summary

- MaxCyte offers a flow electroporation-based platform that is fully scalable for 5E5 cells to 2E11 cells, allowing for production of milligram to multi-gram quantities of protein.
- MaxCyte transient transfection of CHO cells can produce secreted antibody titers over 2.7 g/L with optimization of post transfection culture conditions.
- A variety of CHO cell lines including CHO-S, CHO-K1, CHO EBNA, CHO-K1SV, and CHOZN can be transfected using MaxCyte transient transfection, resulting in higher titers than alternative transfection methods.
- Production scale-up from the MaxCyte STX to the MaxCyte VLX requires no reoptimization while maintaining transfection performance.
- MaxCyte transient transfection results in quality antibodies with glycan profiles similar to a reference antibody produced from stable production cultures. The post transfection feeding protocol had a significant impact on glycan profiles.

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

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