Consistent Antibody Quality and Glycosylation Patterns Support the Use of MaxCyte's Electroporation-based Transfection for Biotherapeutic Product Development.

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Abstract

Companies are turning to transient production of antibodies during early development to delay stable cell line generation, accelerate timelines, and reduce costs. A key factor for the success of this approach is a high degree of similarity between antibodies produced transiently and stably. In this poster, data are shown including transfection performance, scalability, protein quality, and glycosylation analysis, that support the unique ability of MaxCyte's Scalable Transfection technology to accelerate early to mid-stage biotherapeutic development via the transient production of gram-scale quantities of antibodies. Specifically, we will present data on protein quality and glycosylation patterns for antibodies and Fc fusion proteins produced transiently using MaxCyte electroporation and stably produced materials. Reproducibility of MaxCyte's transient expression system will be highlighted, and analytical data on transiently and stably produced proteins will be shared to demonstrate that transiently produced proteins mimic product qualities of stably produced proteins. In addition, long term stability of titers and protein quality in stable cell lines generated via MaxCyte electroporation will he presented. In summary, we demonstrate that transient protein production via MaxCyte transfection can be used to delay stable cell line generation while maintaining the integrity of candidate selection during biotherapeutic development.

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

CHO-based Production of High Quality Proteins

CHO-S: Quality Bispecific Antibody Production

MaxCyte Transfection 20x Higher Titers Than Lipid Transfection

Transfection Method	Expression (purified protein)	%HMW	%Monomer
MaxCyte STX	**173 .0 mg/L**	5.6	94.3
Lipid Reagent	7.3 mg/L	7.2	92.8

STX-produced diabody: Protein A capture & chromatography data

L = lysate
NB = not bound
E = eluate

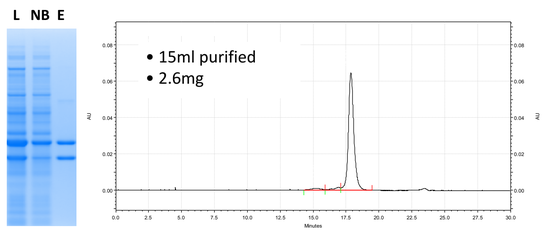
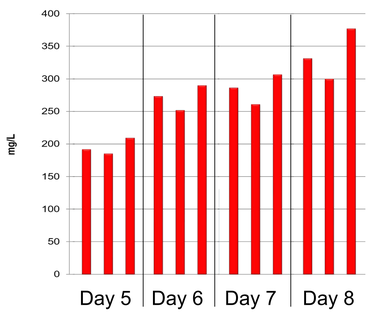


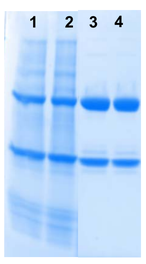
Figure 1: Production of Quality Bispecific Antibodies. CHO-S cells were transfected via static electroporation with a bicistronic expression plasmid encoding the components of a bispecific diabody. Total secreted diabody concentrations were measured using ELSA. Diabody titers were more than 20-fold higher using MaxCyte electroporation compared with a lipid-based transfection reagent. Analysis of purified proteins showed that nearly 95% of the MaxCyte-produced protein was in a monomeric form.

CHOZN®: Gel Analysis of Protein Quality and Titer

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

(via ForteBio-Octet interferometry)





All samples are from day 8

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

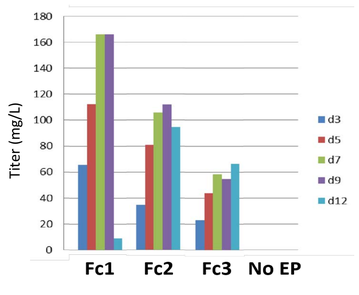
1. Sample 1: unpurified
2. Sample 3: unpurified
3. Sample 1: prot. A pure 10/10
4. Sample 3: prot A pure 10/10

Figure 2: Titers & SDS PAGE analysis of transiently expressed IgGs. CHOZN® cells were electroporated using the MaxCyte STX – three independent runs. Conditioned media samples were collected on days 5-8 and analyzed for IgG titers. Samples from day 8 post EP were run on a Novex 4-20% SDS PAGE Tris Glycine gel and stained with Coomassie Blue G-250. Bands of the correct size for hIgG heavy and light chains are clearly evident on a reducing gel loaded with unpurified media samples and Protein A purified samples. No additional bands are evident in the purified samples, indicating good protein quality.

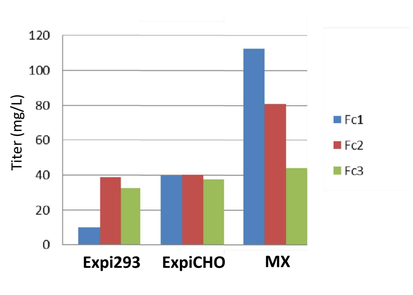
MX CHOK1-SV: Low Degradation Fc Production

MaxCyte Outperforms Expi293 & ExpiCHO

A. MX CHOK1-SV Titers Over Time



B. d5 Titer Comparison: MX, Expi293 & ExpiCHO



C. Fc Quality Over Time: Lower Degradation Using MX Transfection

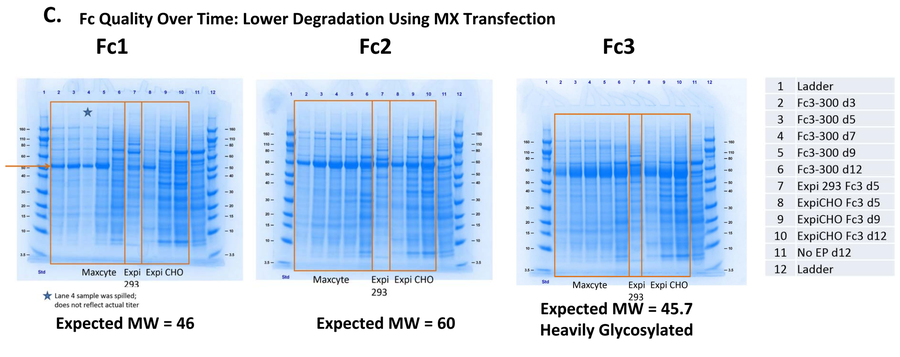


Figure 2: Higher Titers & Less Degradation Using MaxCyte Electroporation. CHOK1-SV cells were transfected with expression plasmids for three Fc molecules via small scale electroporation on the MaxCyte STX. Expi293 & ExpiCHO cells were transfected using their respective standard protocols. Expi293 cultures were generally not viable post d5 (data not shown). A). Media were collected at various time points post transfection and titers determined using an Octet® System. B). Day 5 supernatants for MaxCyte, Expi293 and ExpiCHO transfected cell titers are compared. C). 15ul of sample/lane were run on a reduced 4-12% BT using MES buffer. For 2 of the 3 Fc fragments, MaxCyte titers were significantly higher than both Expi systems. Lower levels of degradation were apparent for MX-produced Fc fragments.

Transient is Consistent with Stably Produced Protein

No Change in Protein Characteristics

Transient & Stable Antibodies Demonstrate Similar Characteristics

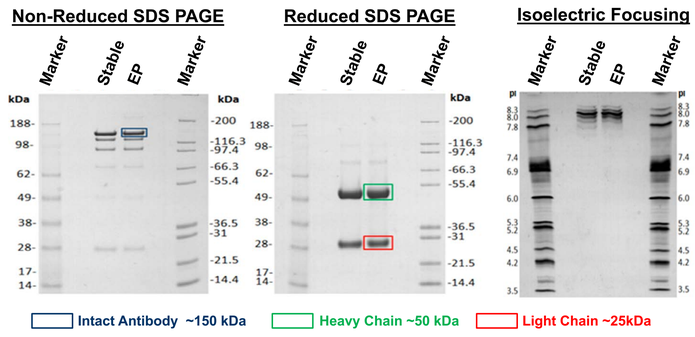
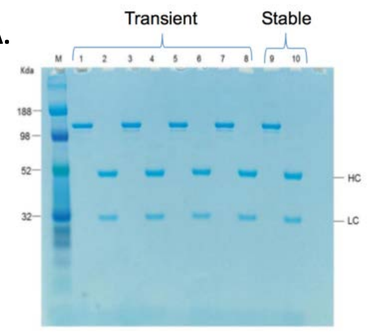


Figure 4. Comparison to Stably Expressed Reference Antibody. CHO suspension cells were transfected by static electroporation with a plasmid encoding IgG heavy and light chain antibody sequences. No differences in size or electrophoretic properties were detectable between the antibody produced via transient gene expression using MaxCyte electroporation (EP) and antibody produced by stably transfected CHO cells (Stable). *Data courtesy of NovImmune*

Equivalent Antibody Quality & Glycosylation

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

A.



SDS PAGE Analysis
Protein A - Purified Antibody (2 µL)

Lane	Sample	Condition
1	S10	NR
2	S10	R
3	S4	NR
4	S4	R
5	S6	NR
6	S6	R
7	S12	NR
8	S12	R
9	S17	NR
10	S17	R

HC: Heavy Chain
LC: Light Chain
NR: Non-reduced
R: Reduced

B.

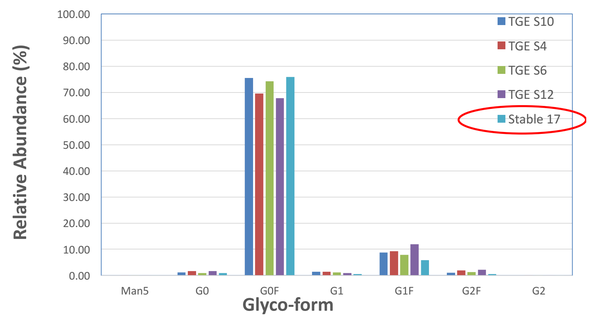
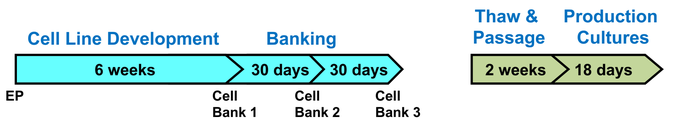
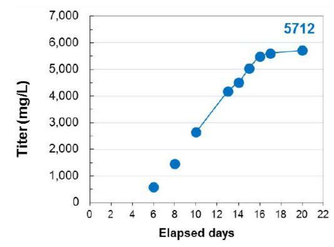


Figure 5: hIgG1 Quality & Glyco-form Comparison - Transient vs Stable Expression. A human IgG molecule was expressed transiently in CHO-S cells via four independent static electroporations with the MaxCyte STX. A stable cell line (S17) was also generated by subjecting transfected cells to antibiotic 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.

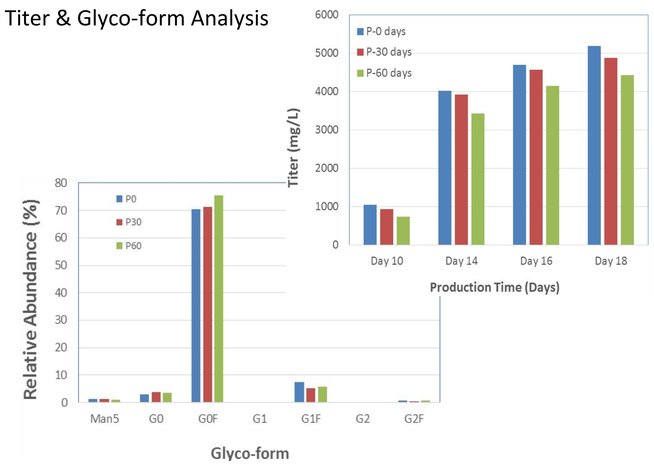
Stability of MaxCyte-produced Cell Lines



A. Top Producing Stable Cell Line 6 weeks Post EP



B. Titer & Glyco-form Analysis



C. Protein Quality

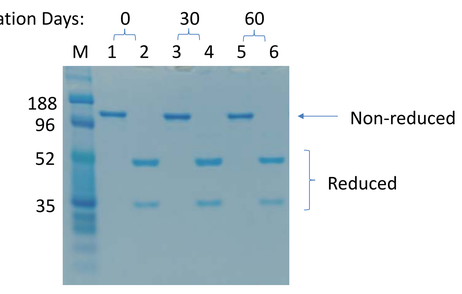


Figure 6: Analysis of IgG Titers, Post-Translational Modifications and Protein Quality of a MaxCyte-produced Stable Cell Line Over Passage. CHO-S cells were subjected to antibiotic selection and limited dilution cloning following static electroporation with the MaxCyte STX. A stable pool was generated within 2 weeks of electroporation. 479 clones were screened following limited dilution cloning. A). The top clone (S17) was selected for production within 6 weeks post transfection. The production was carried out in shake flasks as a fed batch. At day 17 productivity reached over 5.7 g/L. B). Cell banks were generated from S17 at three different time points post selection (day 0, day 30 and day 60). Cells from the three cell banks were thawed and proteins were produced in fed batch cultures. Less than 15% loss in titer was observed after 60 days in culture, and glycoform patterns remained consistent. C). Proteins produced by the three cell banks were purified using Protein A and analyzed via SDS-PAGE analysis. No loss in protein quality was observed.

Summary

- The MaxCyte transfection platform can produce high titers of antibodies and antibody-like molecules using a variety of CHO cell lines, including CHO-S, CHOK1-SV and CHOZN® cells, providing researchers the ability to use their CHO cell line of choice.
- The MaxCyte STX is a highly reproducible means of transient protein production.
- MaxCyte transfected CHO cells rapidly produced higher protein titers compared to lipid-based, Expi293 and ExpiCHO systems.
- Fc fragments produced by Expi293 and ExpiCHO degraded more rapidly in culture than MaxCyte produced fragments. Fc stability did differ significantly from one fragment to another.
- Antibodies produced using MaxCyte electroporation demonstrated similar protein characteristics and glycosylation patterns to antibodies produced by stable cell lines, supporting the use of transiently produced protein in early stage biotherapeutic development.
- 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.
- MaxCyte transfection led to generation of stable cell lines that maintain protein titers, quality and glycosylation patterns over 90 days.

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