Accelerated Generation of Stable Pools & High-yield, Robust Stable Cell Lines Using MaxCyte Electroporation.

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Abstract

MaxCyte's scalable electroporation technology allows gram-scale transient expression of antibodies and other proteins in cell types that are relevant to biomanufacturing. MaxCyte electroporation also offers significant advantages for rapidly generating stable pools and clonally-derived stable cell lines. The high levels of transfection efficiency and cell viability post electroporation can significantly reduce the time needed for cell recovery during selection and create stable pools enriched for high producers as well as shorten the timelines and reduce the labor needed for creating clonal cell lines. In this poster, we detail the rapid generation of stable pools as well as a high-yield stable cell line with a titer of 5.7 g/L within 8 weeks of transfection. We share analytical data to demonstrate that the transiently and stably expressed antibodies are qualitatively equivalent. In addition, data highlighting the stability of antibody titers, quality, and glycosylation profiles of a stable clone over 60 days culture is presented to illustrate the robustness of cell lines generated using MaxCyte electroporation.

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

MaxCyte Transient Transfection for Stable Production

Generation of Quality Stable Pools

7x Higher Antibody Titers Compared to Chemical Methods

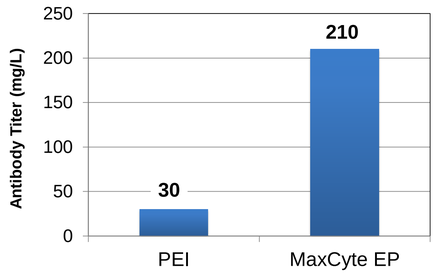


Figure 1: CHO Stable Pool Produce Higher Antibody Titers Following MaxCyte Transfection Compared with Chemical Transfection. CHO cells were cultivated in CD-CHO (Invitrogen) for PEI transfection or CD-Opti CHO (Invitrogen) for MaxCyte electroporation. Cells were pelleted and resuspended at 1.5e6 cells/ml for PEI and 5e6 cells/ml for MaxCyte transfection. For both methods, DNA-to-cell ratio was 1μg/1e6 cells. Secreted antibody titers were measured 9 days post transfection. *Data courtesy of LakePharma.*

MaxCyte Transfected CHO Cells Recover Quickly

8 Days Faster than Chemical-based Methods

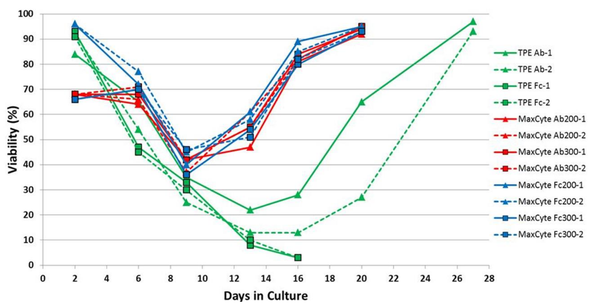


Figure 2: Rapid Recovery of Stable Pools Following MaxCyte Electroporation. Twelve independent CHO cells transfections were performed – four via chemical transfection which are shown in green, and eight via MaxCyte small-scale electroporation, which are shown in red and blue. Half of the transfections for each method were for the expression of a full IgG, while the other half were for the expression of an Fc fragment. Two of the four chemical transfections, specifically those transfected with an Fc expression plasmid, did not fully recover, with almost complete cell loss at day 16 post transfection. Cells from all eight MaxCyte transfection, so those expressing either the IgG or the Fc fragment, all recovered 8 days earlier than the two remaining chemically-transfected cells.

Rapid Generation of Stable Clones

High-producing Clone Identified 6 Weeks post Transfection

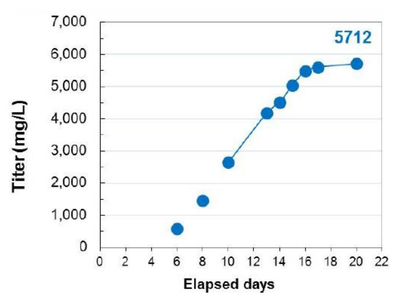


Figure 3. Rapid Stable Cell Line Development Using MaxCyte Flow Electroporation. Humanized mAb DNA was transfected into CHO-S cells using STX technology. Transfected cells were subjected to antibiotic selection and limited dilution cloning. A stable pool was generated within 2 weeks of electroporation. 479 clones were screened following limited dilution cloning. 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.

Transient is Consistent with Stably Produced Protein

No Change in Protein Characteristics

Transient & Stable Antibodies Demonstrate Similar Characteristics

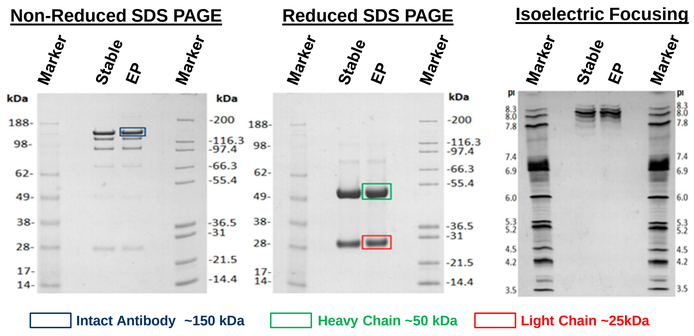
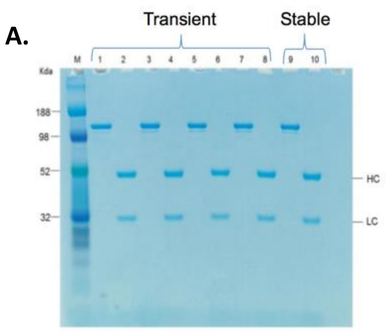


Figure 4. Comparison to Stably Expressed Reference Antibody. CHO suspension cells were transfected by static electroporation with a plasmid encoding IgG heavy and light chain antibody sequences. No differences in size or electrophoretic properties were detectable between the antibody produced via transient 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.

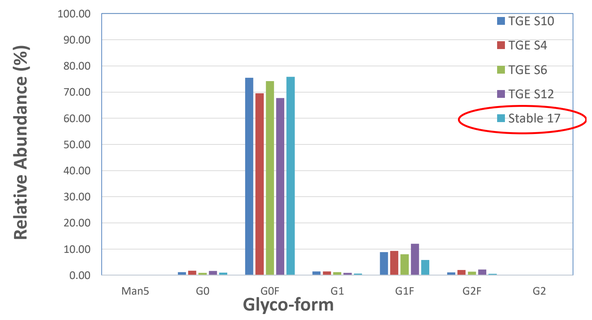
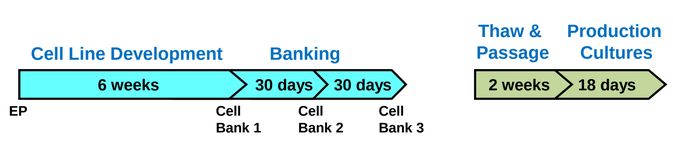
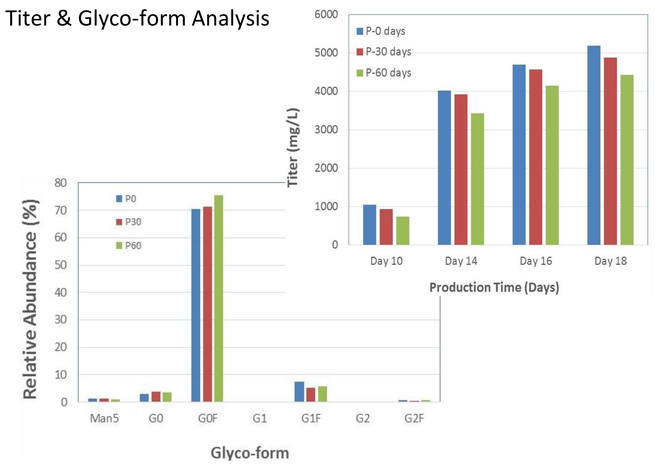


Figure 5: hIgG1 Quality & Glyco-form Comparison - Transient vs Stable Expression. A human IgG molecule was expressed transiently in CHO-S cells via four independent static electroporations with the MaxCyte STX. A stable cell line (S17) was also generated by subjecting transfected cells to antibiotic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



B. Protein Quality

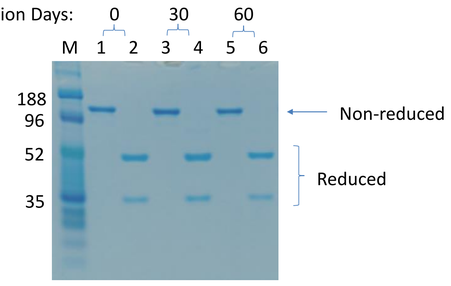


Figure 6: Analysis of IgG Titers, Post-Translational Modifications and Protein Quality of a MaxCyte-produced Stable Cell Line Over Passage. CHO-S cells were subjected to antibiotic selection and limited dilution cloning following static electroporation with the MaxCyte STX. The top clone (S17) was selected for production within 6 weeks post transfection (see Figure 3 for productivity data. A). 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. B). 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

- MaxCyte electroporation generates high quality stable pools faster than chemical-based transfected methods. MaxCyte stable pools recovered over a week faster than chemical transfection.
- 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.
- Antibodies produced using MaxCyte electroporation demonstrate 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.
- MaxCyte transfection led to generation of stable cell lines that maintain protein titers, quality and glycosylation patterns over 60 days.

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