Shortening the Timeline for Drug Development by Enabling Large-Scale TGE in CHO Cells and High Producing Stable CHO Cell Line

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MaxCyte® FLOW TRANSFECTION

Abstract

To reduce early-stage development costs and lower late-stage attrition rates, many biopharmaceutical companies have turned to transient gene expression (TGE) rather than developing stable cell lines for early-stage development work. With the ability to produce gram-level quantities of antibodies, Maxcyte's flow electroporation extends the applicability of TGE to later stages of antibody development. In addition, since the regulatory standard for manufacturing of clinical-grade biotherapeutics remains stable CHO cell line production, MaxCyte's proprietary electroporation technology can greatly shorten the timeline of antibody development by enabling large-scale TGE directly within CHO cells. MaxCyte' flow electroporation technology offers a universal means of fully scalable, high efficiency TGE and is capable of producing multiple grams of antibodies and bi-specific antibodies following a single CHO transient transfection. In the meantime, a high producing stable CHO cell line can be rapidly generated with our technology due to its superior transfection efficiency and cell viability. In this poster, data will be presented demonstrating the TGE ability of MaxCyte flow electroporation to produce antibody titers up to 2.7 g/L. Data also will be presented showing the use of MaxCyte electroporation for creation of stable CHO pools and the rapid generation of high-yield stable cell lines with a titer of 5.7 g/L within 6-8 weeks of transfection.

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® Transfection Systems use fully scalable flow electroporation for rapid, highly efficient transfection with very high cell viability post transfection. Transfected cells support multi-gram scale production of antibodies and proteins for efficient biotherapeutic development.

Transfection of CHO Cells

Highly Efficient DNA Loading in CHO Cells

>95% Viability and Transfection Efficiency

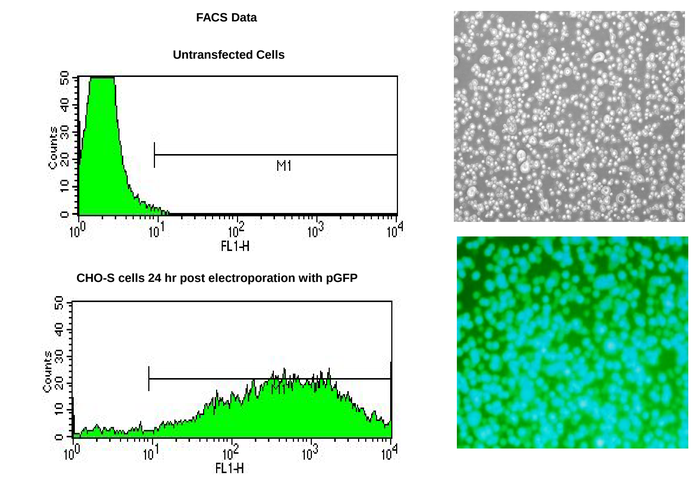


Figure 1. Greater than 95% CHO cell Transfection Efficiency and Cell Viability Using MaxCyte Transient Transfection. CHO-S cells were transfected with a plasmid encoding green fluorescent protein (2 µg DNA/1E6 cells) using small-scale (static) electroporation on the MaxCyte STX. GRP expression and viability were measured by flow cytometry (FACS) 24 hours post electroporation.

Multi-Gram Scale Antibody Production by TGE

2.7 g/L Achieved in <3 Weeks

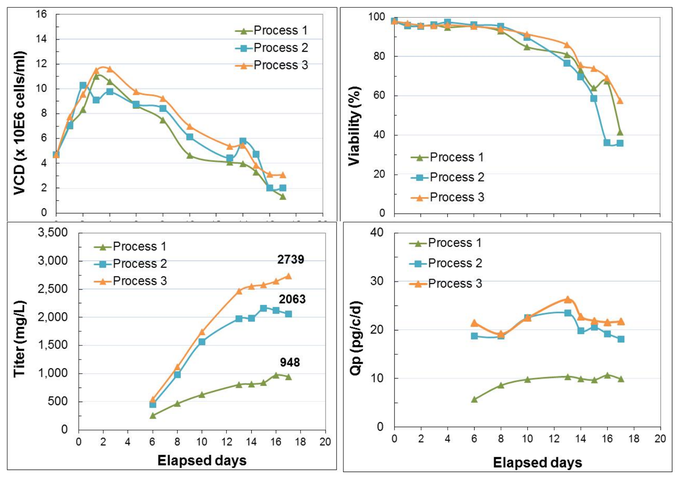


Figure 2. Transient Expression of hIgG1 antibody in MaxCyte EP Transfected CHO-S cells. The same transfected cells were in different production processes. Further optimized process (process 3) can reach 2.7 g/L as a fed batch. Titer was verified by both Elisa and Protein A capture assays.

MaxCyte-Transfected CHO Cells Recover Quickly

7 Days Faster than Other Methods

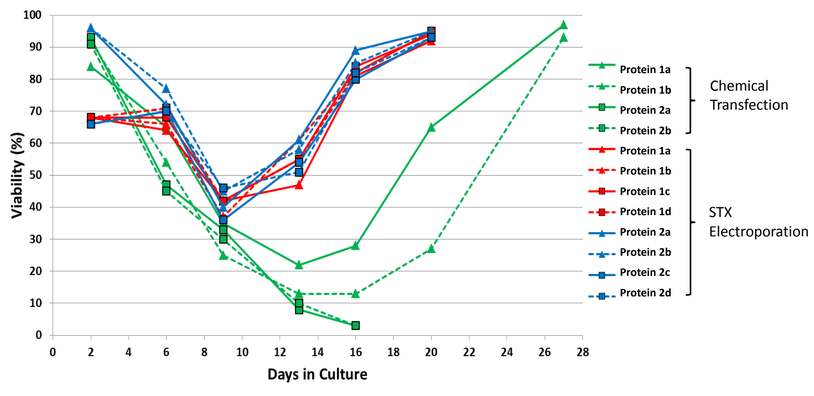


Figure 3. Rapid Generation of Stable Pools Following Chemical Selection of Transfected Cells. CHO cells were transfected with two different protein expression plasmids via static electroporation in OC-400 PAs or by a reagent-based method. Selection was applied one day post transfection. Cells transfected via the SXT recovered more quickly from selection compared to cells transfected using a chemical transfection reagent.

Stable Cell Line Generation

Rapid Generation of Stable Pools

3X Higher Antibody Titers Compared to Chemical Methods

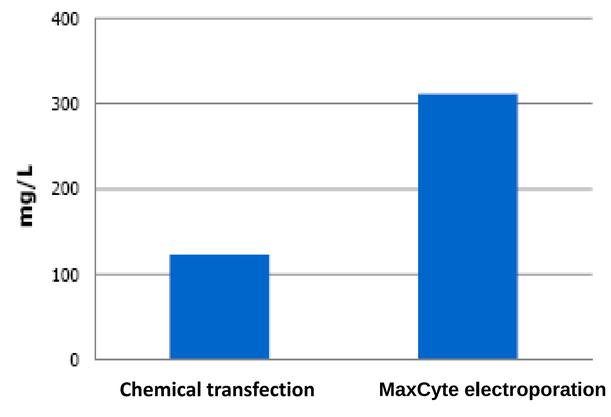


Figure 4. CHO Stable Pool Production Using MaxCyte or Chemical Transfection. CHO cells were cultivated in CD-CHO (Invitrogen) for PEI transfection or CD-OPTI CHO (Invitrogen) for MaxCyte EP. Cells were pelleted and resuspended at 1.5E6 cells/mL for PEI or 5E6 cells/mL for MaxCyte EP. For both methods, DNA-to-cell ratio was 1 µg/1E6 cells. Secreted antibody titers were measured 9 days post transfections. Data courtesy of LakePharma.

Antibody Titers by Rapidly Generated Stable Cell Line

Stable Cell Line Quantities Achieved in 3 Weeks

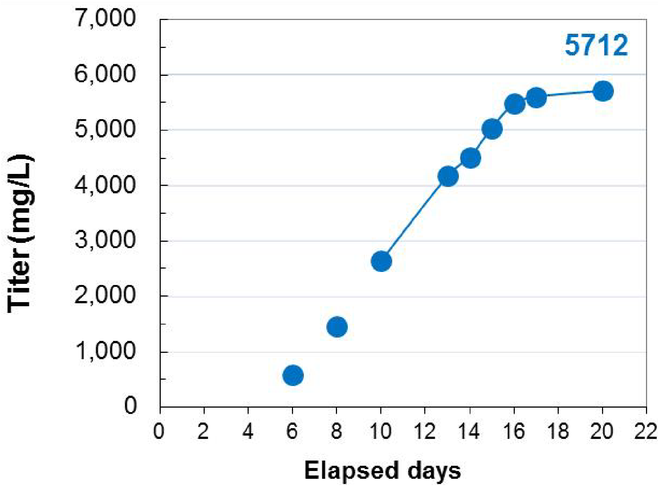
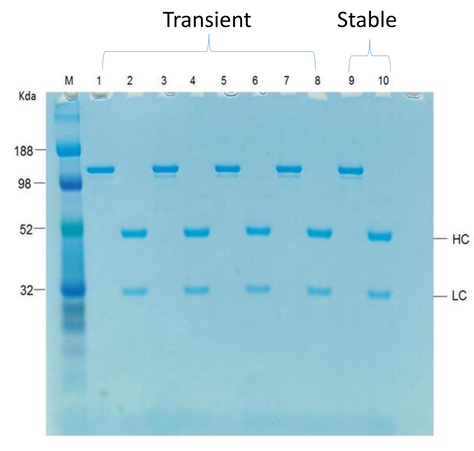


Figure 5. A Fed Batch of a Stable Clone. A stable pool was generated within two weeks of electroporation, and,479 clones were screened following limited dilution cloning. The top clone was selected for production within 6 weeks post transfection. The production was carried out in the shake flask as a fed batch. At day 17, productivity can reach 5.5 g/L and results were verified by both Elisa and Protein A capture assays.

Stable vs. Transient Expression Are Equal

No Difference Seen in Antibody Quality



SDS-PAGE Analysis of Protein A-Purified MaxCyte mAbs.

Lane 1: S10 Non-reducing, 2 µg;
Lane 2: S10 Reducing, 2 µg;
Lane 3: S4 Non-reducing, 2 µg;
Lane 4: S4 Reducing, 2 µg;
Lane 5: S6 Non-reducing, 2 µg;
Lane 6: S6 Reducing, 2 µg;
Lane 7: S12 Non-reducing, 2 µg;
Lane 8: S12 Reducing, 2 µg;
Lane 9: S17 Non-reducing, 2 µg;
Lane 10: S17 Reducing, 2 µg;
HC: Heavy chain; LC: Light chain.

Figure 6. SDS-PAGE Gel Analysis of hIgG1 Products from TGE and Stable Expressions. Sample 17 is the material from stable cell line. All others are transient expression materials. Data showed all have similar quality.

Summary

- MaxCyte offers a single flow electroporation-based platform that is fully scalable from 5E5 cells to 2E11 cells allowing for production of milligram to multi-gram quantities of proteins.
- MaxCyte flow electroporation can result in greater than 95% CHO cell transfection efficiency and cell viability
- MaxCyte transient transfection of CHO cells can produce secreted antibody titers **over 2.7 g/L** with optimization of post transfection culture conditions.
- Rapid and efficient stable cell line development process shows a high stable producer with **5.7 g/L.**
- Antibodies produced by transiently transfected CHO cells are indistinguishable from those produced by stable cell lines based on SDS PAGE.
- Due to the high cell viability of MaxCyte flow electroporation, cells may recover 7 days faster than other methods form chemical selection.
- MaxCyte electroporation produced 7X the antibody titers compared to a chemical transfection method.
- The quality of the antibodies produced using MaxCyte flow electroporation is consistent with that of antibodies produced from stable cell lines.

Benefits of MaxCyte Flow Electroporation

- The ability to transfect a large number of cells (2E11) allows for both the generation of antibodies for screening along with clonal selection from the same cell population, saving time and resources and improving consistency.
- MaxCyte electroporation enables research and development to be done in a biologically relevant cell, minimizing risk of irrelevant candidates being put forward.
- Stable cell lines can be produced faster with MaxCyte electroporation, enabling speed to market.
- MaxCyte electroporation uses cells at a high density, enabling reductions in process volume to 20-25% of that required for reagent-based transfection methods. Significant process and time savings can be realized from reduced media and feed requirements, smaller reaction vessels, simplified and shorter filtration/purification cycles, and impacts on other unit operations.
- The ability to transfect a large number of cells (2E11) means clonal selection can be started on a small fraction, and the rest used to generate protein or data immediately, saving time and resources.
- Electroporation and scale-up protocols are optimized and computer controlled, with reproducible results from day-to-day and operator-to-operator. There is no need for reoptimization of reagents or upstream/downstream scale changes, saving time and improving productivity by allowing more campaigns with the same amount of resources.

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