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 the production of high quality antibodies with a high degree of similarity to stably produced proteins. MaxCyte’s flow-electroporation based delivery platform offers significant advantages across the therapeutic development pipeline by transiently producing milligram to gram quantities of protein and rapidly generating stable pools and stable clones using cell backgrounds that are relevant to biomanufacturing. In this poster, data are shown including transfection performance in multiple CHO cell lines as well as protein quality and glycosylation profiles that demonstrate the similarity of transiently stably produced antibodies. Furthermore, we demonstrate that 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. These features shorten the timelines and reduce the labor needed for creating clonally-derived cell lines. In summary, we illustrate that MaxCyte Flow Electroporation Technology can expand the footprint of transient transfection by delaying stable cell line generation while maintaining the integrity of candidate selection thereby accelerating early to mid-stage biopharmaceutical development.

MaxCyte Outperforms PEI of CHO-K1SV Cells

Large Increase in Mouse & Human IgG Expression

Figure 1: High Performance Transfection of a Variety of CHO Cell Lines. CHO2N, CHO1K5V, CHO37, and CHO DG44-derived cells were transfected with a GFP expression plasmid via MaxCyte STX™ FACS analysis was performed 24-48 hrs post electroporation.

Figure 2: PEI vs. MaxCyte Comparison for Antibody Expression in CHO-K1SV Cells. CHO K1SV cells were transfected using PEI or the MaxCyte STX for a human or mouse IgG. Two DNA were used using MaxCyte electroporation. All MaxCyte transfected cells were shifted to 32°C post electroporation. Half of PEI samples were shifted to 32°C while half remained at 37°C post transfection. Transfection efficiency was determined by antigen binding. The ELISA of transfection conditioned media. Antibody titers of day 7 media were determined for all transfection and post transfection conditions. Inset table shows actual antibody levels depicted in the associated graph.

Consistent Antibody Quality & Glycosylation

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 (GE-857 cell 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.

Figure 4: MaxCyte STX™ and MaxCyte VLX™ Transfection Systems use scalable Flow Electroporation Technology for rapid, highly efficient, highly consistent transfection.

CHO EBNA & 293 EBNA: PEI versus MaxCyte

Superior Antibody Expression Using the MaxCyte STX

Figure 5: SDS-PAGE Analysis of transfectionally produced IgGs. CHO37, CHO2N cells were transfected using the MaxCyte STX™ – three independent runs. Samples from day 7 were collected on days 5 & 7 and analyzed for antibody expression. Antibody bands were present in both MaxCyte STX™ and PEI Control runs. However, antibody bands were sharper and denser in the MaxCyte STX™ runs.

CHO2N: Gel Analysis of Protein Quality & Titer

Figure 6: Glyco-Quality & Glyco-form Analysis - Transiently Stable Expression. A human IgG molecule was expressed transiently in CHO-D cells via four independent electroporation runs on the MaxCyte STX™. Antibody Elisa G2F MaxCyte Flow Electroporation™ Technology outperforms other transfection methods including chemical methods such as PEI resulting in higher, more consistent antibody production.

CHO cell lines exhibit strong human and mouse IgG production following MaxCyte electroporation. CHO-S cells exhibited titers >2.7 IgG following lead optimization enabling rapid, gram-scale production of antibodies.

Antibodies transiently produced using MaxCyte electroporation demonstrate similar protein characteristics and glycosylation patterns to antibodies produced by stable cell lines, supporting the use of transiently produced proteins in early-stage discovery efforts allowing the delay of stable cell line generation and thereby reduced costs.

MaxCyte electroporation is fully scalable allowing a single large-scale production for transient protein production and simultaneous generation of stable clones.

The high cell viability and transfection efficiencies of MaxCyte’s delivery platform generate quality stable pools faster than chemical-based methods.

Max 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 antibody selection which achievement for high yield clones. Fewer than 500 clones needed to be screened to identify a high yield cell line.

MaxCyte Delivery Platform for Cell Engineering

Figure 8: Rapid Stable Cell Line Development Using MaxCyte Flow Electroporation. A stable pool of CHO cells expressing an IgG was generated within two weeks of electroporation. A 479 clones were screened following limited dilution cloning. The top clone (clone #17) was selected for production in 6 weeks post transfection. This cultured cell line was subjected to MA6 selection beginning on day of electroporation. STX transfected cells recovered from selection was quicker than compared to cells transfected with the same plasmid using PEI.

Summary

• The MaxCyte delivery platform consistently results in high transfection efficiency for a variety of CHO cell lines providing researchers the flexibility to use the CHO cell line of choice throughout their biopharmaceutical development activities.

• MaxCyte Flow Electroporation™ Technology outperforms other transfection methods including chemical-based methods such as PEI resulting in higher, more consistent antibody production.

• CHO cell lines exhibit strong human and mouse IgG production following MaxCyte electroporation. CHO-S cells exhibited titers >2.7 IgG following lead optimization enabling rapid, gram-scale production of antibodies.

• Antibodies transiently produced using MaxCyte electroporation demonstrate similar protein characteristics and glycosylation patterns to antibodies produced by stable cell lines, supporting the use of transiently produced proteins in early-stage discovery efforts allowing the delay of stable cell line generation and thereby reduced costs.

• MaxCyte electroporation is fully scalable allowing a single large-scale production for transient protein production and simultaneous generation of stable clones.

• The high cell viability and transfection efficiencies of MaxCyte’s delivery platform generate quality stable pools faster than chemical-based methods.

• 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 antibody selection which achievement for high yield clones. Fewer than 500 clones needed to be screened to identify a high yield cell line.

Rapid CHO Cell Recovery

Figure 7: Rapid Recovery of Stable Pools Following MaxCyte Electroporation. CHO cells (proprietary cell line) were transfected via static electroporation in DC-400 process; assembled with varying concentrations of an antibody expression plasmid. Transfection were cultured in shake flasks and subjected to MA6 selection beginning on the day of electroporation. STX transfected cells recovered from selection was quicker than compared to cells transfected with the same plasmid using PEI.

• Rapid, High-Yield Stable Cell Generation

Stable Cell Lines Generated in 6 Weeks

A. High-Producing Stable Clone Identified

B. Process Development Boosts Stable Clone Production

Figure 9: Rapid Recovery of Stable CHO Cell Lines Using MaxCyte Flow Electroporation. A stable pool of CHO cells expressing an IgG was generated within two weeks of electroporation. A. 479 clones were screened following limited dilution cloning. The top clone (clone #17) was selected for production in 6 weeks post transfection. B. The production culture 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.