Abstract

Researchers have looked to recombinant technologies to develop innovative types of vaccines and new cell culture-based means of production that offer shorter lead times and greater production flexibility while maintaining vaccine safety. Transient transfection offers a means of rapidly producing an array of proteins, including antibodies, vaccines, viral vectors, and virus-like particles (VLPs). Although a variety of transient transfection methods are available, most do not meet the requirements of scalability, consistency, and cell type flexibility for use in vaccine development and manufacturing. MaxCyte’s electroporation-based delivery platform reproducibly transfects a broad range of biorelevant adherent and suspension cell types with high cell viabilities and transfection efficiencies using single-use processing assemblies for GMP “plug-and-play” production of recombinant proteins and vaccines. In this poster we present data for large-scale production of antibodies, recombinant antigens, VLPs, and lentiviral vectors using the MaxCyte STX® Scalable Transfection System. Data are presented for high-efficiency transfection of cells commonly used in protein production including CHO, HEK293, and insect cells—without the use of baculovirus—with a timeline of just a few days from plasmid to gram quantities of protein.

Expression of Multiple Protein Types Using MaxCyte’s Delivery Platform

High Cell Viability Leads to Strong HIV Antigen Expression

<table>
<thead>
<tr>
<th>Days Post Transfection</th>
<th>MacCyte Large-scale Small-scale PEI</th>
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<tbody>
<tr>
<td>1</td>
<td>27.8 %</td>
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<tr>
<td>7</td>
<td>70.2 %</td>
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<tr>
<td>10</td>
<td>95.2 %</td>
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Figure 2: Cell Viability and Protein Titer Data Following Transfection of CHO Cells with an HIV Envelope Protein Expression Plasmid. CHO-S cells were transfected with a gp140 expression plasmid via small scale, static EP (867 cells) and large scale, flow EP (246 cells). Transfected cells were incubated in shake flasks at the same density and cultured for 10 days. Cell viabilities at large scale EPs yielded consistent titers and exhibited high viabilities. Viabilities and titers from the electroporated cells were much higher than corresponding values for a customer’s optimized PEI process.

Viral Vector Production: Seamless Scalability for Rapid Manufacturing

Consistent Large-scale Lentivirus Manufacturing Using Suspension Cells

Seamless Lentivirus Scalability - MaxCyte STX to VLX

High Titer AAV Production in HEK Cells

Figure 3: Post Electroporation Culture Process Optimization Produces CHO-S Antibody Titerers 2.7 g L-1 CHO-S cells were transfected with an antibody expression plasmid (1ug DNA/1E6 cells) via small scale electroporation on the MaxCyte STX. Cells were plated at approximately 456 cells/mL post electroporation. Transfected cells were cultured in media with different additives and culture conditions. Titer was verified by both ELISA and Protein A capture assays. The optimized process produced antibody titers of 2.74 g L-1 at day 17 post EP as a fed batch.

Figure 6: Scale Up of Lentiviral Vector Production from Small-Scale to Large-Scale Production Using the MaxCyte Platform. Suspension-adapted HEK 293FT cells were suspended in MaxCyte® buffer at 1E6 cells/mL. A mixture of plasmids encoding lentivector components was added to the cells (40 ug DNA/1E6 cells), and cells were transfected to sterile OC-400, CL-2 and VLXD processing assemblies. Cells in the OC-400 and CL-2 were transfected by static and flow EP, respectively, using the STX instrument; cells in the VLXD were transfected by flow EP on the VLX. Lentiviral titers were measured after 24-44 hrs in culture. Normalized titer data show seamless scalability of the MaxCyte transfection process.

Figure 7: Production of AAV in HEK Cells. (A) Adherent HEK cells were transfected with three plasmids encoding AAV vector components (GFP transgene) via static electroporation using the MaxCyte STX. (B) Nearly 100% of the transfected cells exhibited robust transgene expression 48 hours post electroporation. (C) High AAV titers were detected in cell pellets via qPCR analysis.

Summary

- MaxCyte’s electroporation-based delivery platform is fully scalable from 5E5 cells to 2E11 cells allowing for production of milligram to multi-gram quantities of proteins, viral vectors, and multi-protein complexes such as VLPs.
- MaxCyte electroporation is high performance means of transiently transfecting adherent and suspension cell lines commonly used during vaccine development and production such as CHO, HEK, and insect cells.
- MaxCyte transfection rapidly produces a variety of proteins including antigens, antibodies, lentiviral vectors, and VLPs more efficiently than chemical transfection methods and baculovirus expression systems.
- MaxCyte transfection of CHO cells can produce secreted antibody titers >2.5 gram/L with optimization of post transfection culture conditions.
- Insect cells rapidly express recombinant proteins, including VLPs, at high efficiency following MaxCyte electroporation, eliminating the need for baculovirus use.
- MaxCyte electroporation results in high-performance transfection of suspension-adapted HEK cells allowing for large-scale, highly reproducible production of viral vectors.
- Production scale-up from the MaxCyte STX to the MaxCyte VLX is seamless — maintenance of transfection performance without the need for reoptimization.

MaxCyte Delivery Platform

The MaxCyte® STX™ and MaxCyte VLX™ 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

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