Universal, Fully Scalable Transfection Platform for Production of Complex or Difficult-to-Express Proteins: Superior Performance over Other Transfection Methods & Expression Systems.

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Abstract

Biotherapeutic development often requires the production of gram level quantities of recombinant proteins. Transient transfection offers a means of rapidly expressing a variety of proteins including antibodies, antibody-like molecules, biochemical targets of interest, 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. MaxCyte's proprietary flow electroporation technology produces proteins from a variety of adherent and suspension cell types faster than creation of stable cell lines. In this poster we describe large scale electroporation using the MaxCyte STX Transfection System for the production of several antibodies, including bi-specific antibodies, VLPs and a lentiviral vector. Data will be presented for high efficiency transfection of cells commonly used in protein production including CHO, HEK293 and insect cells. Results from comparisons to other transient transfection technologies such as lipid reagents and PEI demonstrate the superior utility and quality of MaxCyte electroporation.

MaxCyte Transient Transfection Systems

MaxCyte STX®

5E5 Cells in Seconds
Up to 1E10 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
- Streamlined scalability requiring no re-optimization

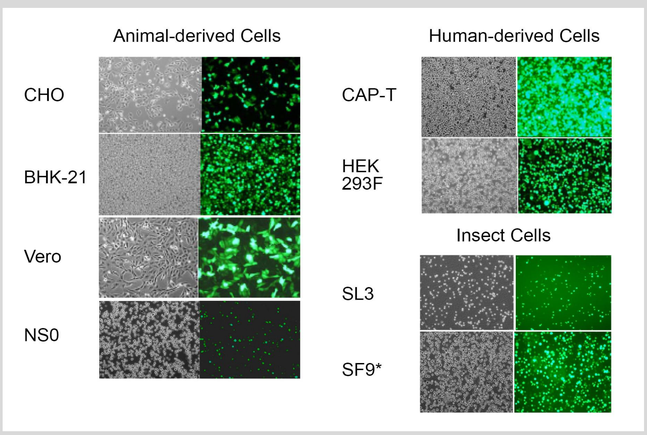

Figure 1. High Efficiency Transfection of Cell Types Commonly Used for Protein Production. Various cells were transfected with 2 µg/1E6 cells of pGFP DNA using the appropriate MaxCyte STX protocol. Cells were examined for GFP expression using fluorescence microscopy 24 hrs post electroporation.

Summary

- MaxCyte electroporation enables rapid transient transfection producing high cell viabilities & transfection efficiencies for a variety of commonly used cell lines include human-, animal-, and insect-derived cells.
- MaxCyte electroporation lead to higher level expression of multiple antibodies and recombinant proteins when compared directly with chemical and lipid-based transfection.
- A breadth of biotherapeutic molecules can be expressed at high levels including antibodies, bi-specific antibodies, recombinant proteins, and viral proteins using MaxCyte electroporation.
- MaxCyte transient transfection of CHO cells leads to production of consistently high titers of antibodies & antibody-like molecules.
- MaxCyte transfection produces quality antibodies at high titers. Secreted titers were over 20x greater than those produced using lipid-based transfection.
- Insect cells rapidly express recombinant proteins at high efficiency following MaxCyte electroporation, eliminating the need for baculovirus use.
- Recombinant protein expression levels following insect cell electroporation exceed those of chemically transfected cells and of a reference stable cell line.

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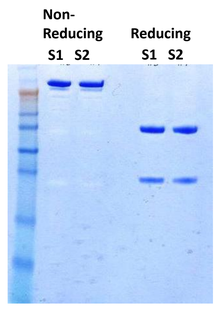
Antibody and Antibody-like Molecule Production

Reliable, High Titer Antibody Expression

Rapid production of quality monoclonal antibodies



	Titer Based on Protein A Capture
Sample 1	456 mg/L
Sample 2	496 mg/L

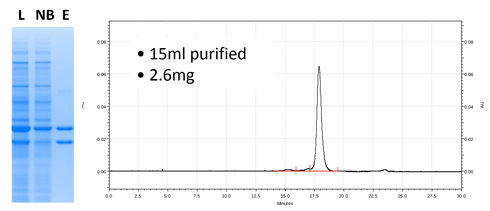
Figure 2. CHO-based Antibody Production with Titers Consistently Exceeding 450 mg/L. CHOS cells were transfected with bicistronic expression plasmids encoding human IgG1 heavy and light chain proteins in two independent small scale (static) electroporations using OC-400 processing assemblies. After nine days of culture in shake flasks, antibodies were purified from 25 mL of conditioned media via protein A capture. Titers based on the quantities of isolated protein exceeded 400 mg/L in both transfections. Gel analysis revealed an absence of high molecular weight protein aggregates and showed normally sized heavy and light chain molecules with no evidence of degraded or truncated proteins.

Production of Quality Bispecific Antibodies

MaxCyte Transfection: 20x greater production than lipid-based transfection

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

STX-produced diabody: Protein A capture & chromatography data



L = lysate
NB = not bound
E = eluate

Figure 3. Production of Quality Bispecific Antibodies. CHOS cells were transfected via static electroporation in an OC-400 processing assembly with a bicistronic expression plasmid encoding the components of a bispecific diabody. Total secreted diabody concentrations were measured using ELISA on various days post transfection. Diabody titers were more than twenty fold higher using MaxCyte electroporation. Analysis of purified proteins showed that most all of the MaxCyte produced protein was in a monomeric form.

Insect Cell Protein Expression

High Efficiency Electroporation of Sf9 Cells

Rapid Protein Expression

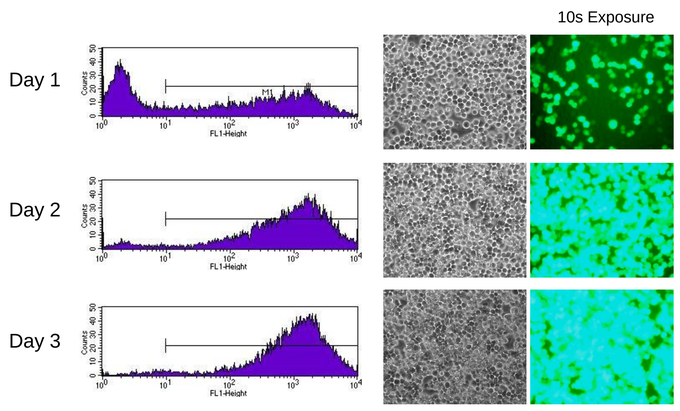
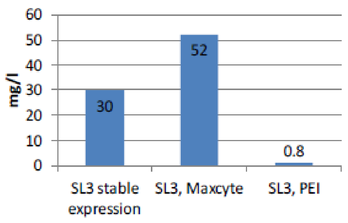


Figure 4. Kinetics of GFP Expression Following Electroporation of Insect Cells. SF9 cells were transfected with a GFP plasmid (baculovirus-based vector) at 2µg/1E6 cells using static electroporation. GFP expression was assessed using FACS analysis and fluorescence microscopy at Days 1, 2, and 3 following electroporation. High transfection efficiencies enable the use MaxCyte electroporation for insect cell protein expression eliminating the need to use baculovirus expression systems.

Increased Expression in SL3 Cells

MaxCyte Transfection: Stronger expression than stable cell line or PEI transfection



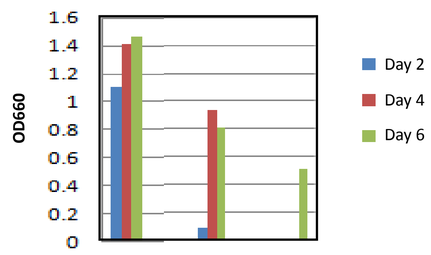
Stable expression	Transient expression	
	MaxCyte STX	PEI
SL3 cells	SL3 cells	SL3 cells
5 L (several batches)	0.25 L	0.25 L

Figure 5. Higher SL3 Expression Following Electroporation vs. PEI and Stable Cell Expression. SL3 insect cells were transfected via static electroporation with a plasmid encoding a secreted protein expressed via a baculovirus-derived promoter. Secreted protein titers generated with MaxCyte transfected cells greatly exceeded titers produced by PEI transfected cells, and surpassed titers from a stably transfected cell line expressing the same protein.

Recombinant Antigens & Viral Proteins

Production of Antigens from Multiple Species

MaxCyte Transfection: Higher, more rapid antigen expression



Species	Bovine	Feline	Feline
Transfection Method	STX	STX	Lipid-based

Figure 6. MaxCyte Transfection Outperforms Lipid-based Transfection for Expression of Antigens from Multiple Species. HEK 293F cells were transfected via electroporation in OC-400 processing assemblies with expression plasmids encoding bovine and feline proteins to generate controls for immunoassays. ELISA analysis of conditioned media samples revealed increasing titers for at least six days with the bovine protein. Although titers decreased slightly by day 6 with the feline protein, volumetric productivity of MaxCyte transfected cells exceeded titers generated via lipid-based transfection.

More Efficient Production of Viral Proteins

MaxCyte Transfection: Higher expression of viral protein than chemical-based method

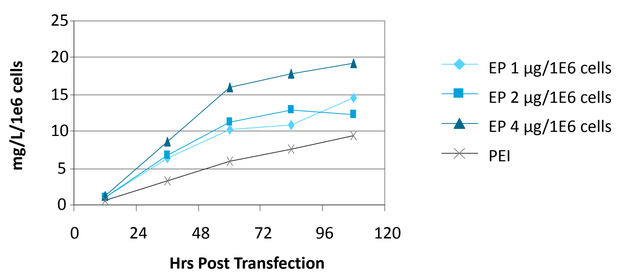


Figure 7. Superior Production of a Viral Protein using MaxCyte Electroporation. HEK 293F cells were transfected with varying concentrations of a viral coat protein expression plasmid using the MaxCyte STX or using an optimized polyethyleneimine (PEI) method. Transfected cells were cultured for approximately 5 days. Culture media was collected without replacement at various times post transfection and protein titers measured via ELISA.