

# Protein Production in Insect Cells: Flow Electroporation, a Superior Alternative to Baculovirus Expression.



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## Abstract

Insect cells are used for the industrial manufacturing of many products that are currently in clinical trials or already available on the market for veterinary and human applications. Importantly, insect cells provide for high levels of protein expression with protein folding and post-translational processing similar to that of mammalian cells. In addition, insect cells present several advantages over mammalian cells for large-scale protein biomanufacturing, including availability of suspension cell lines, relative ease of culture, growth in serum- and protein-free media, and the overall speed of protein production. Unlike other conventional transient transfection technologies or baculovirus expression systems, electroporation from MaxCyte requires no specialized constructs, viral stock production, engineered cells, media additives, or chemical reagents and allows progression from plasmid to high yield protein production within days. MaxCyte scalable electroporation reproducibly (co)transfects cells producing high-transfection efficiencies and cell viabilities for a variety of cells commonly used for protein production including CHO, HEK, Vero, NS0, and insect cells, and has the capacity to transfect up to 2E11 cells in less than 30 minutes. In this poster data are presented showing the rapid production of recombinant proteins and VLPs following transfection of SF9, SF21, and SL3 cells using the MaxCyte STX® Scalable Transfection System. Results from comparisons to baculovirus expression and PEI transient transfection demonstrate the superior quality and speed of MaxCyte electroporation for large-scale, insect cell protein production.

## MaxCyte Transient Transfection Platform



**MaxCyte STX®**  
5E5 Cells in Seconds  
Up to 1E10 Cells in <30 Min.



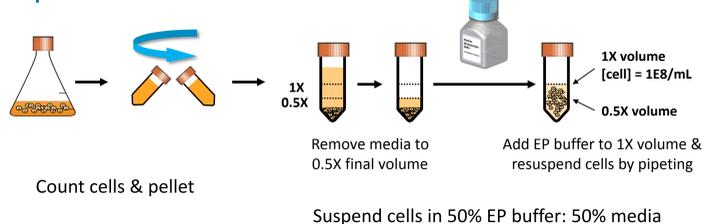
**MaxCyte VLX®**  
Up to 2E11 Cells in <30 Min

The MaxCyte STX® and MaxCyte VLX® Transient Transfection Systems use fully scalable flow electroporation for rapid, highly efficient transfection.

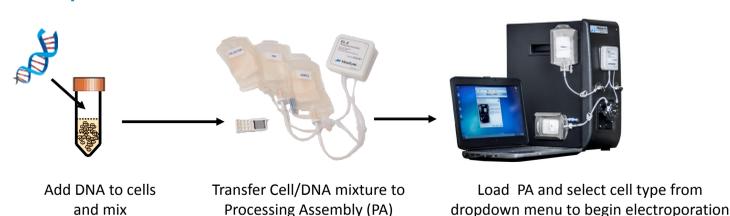
- High efficiency & high cell viability
- Broad cell compatibility
- Streamlined scalability requiring no re-optimization

## Simple, 3-step Insect Cell Electroporation Process

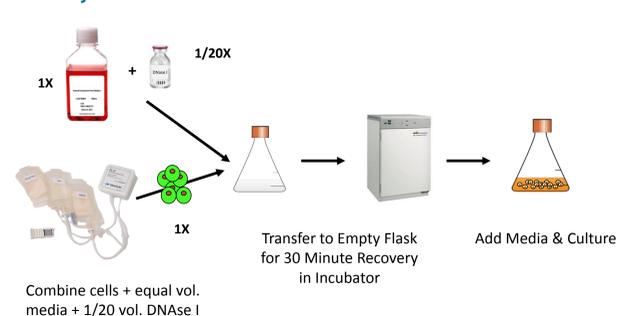
### Prepare Cells



### Electroporation



### Recovery & Culture

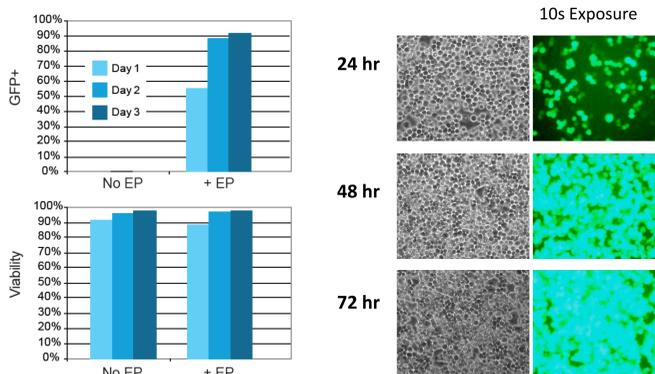


**Figure 1. 3-step, Easy-to-perform Insect Cell Electroporation Process.** Small- and large-scale MaxCyte electroporation follow the same basic 3-step process: cell harvesting, electroporation, and a brief post electroporation recovery. The MaxCyte STX and MaxCyte VLX come pre-loaded with cell-type specific protocols making electroporation a simple push-button operation.

## Insect Cell Transfection

### High Efficiency Electroporation of Sf9 Cells

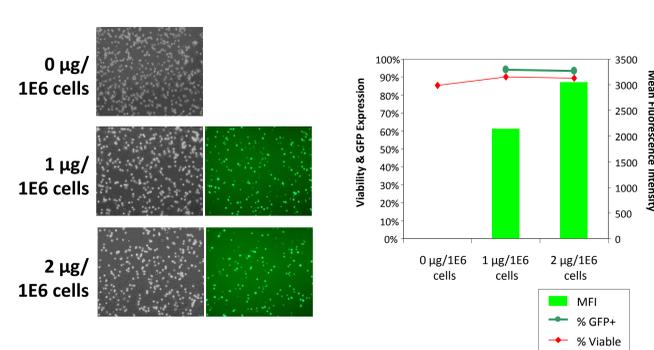
Rapid, High-level Protein Expression



**Figure 2. Kinetics of GFP Expression Following Electroporation of Sf9 Insect Cells.** Sf9 cells were transfected with a GFP plasmid (expression driven by AcNPV baculovirus-derived enhancer and promoter elements) at 2 µg/1E6 cells. GFP expression was assessed using FACS analysis and microscopy at Days 1, 2, and 3 following electroporation. High-transfection efficiencies enable the use of MaxCyte electroporation for insect cell protein expression, eliminating the need to use baculovirus gene transfer systems.

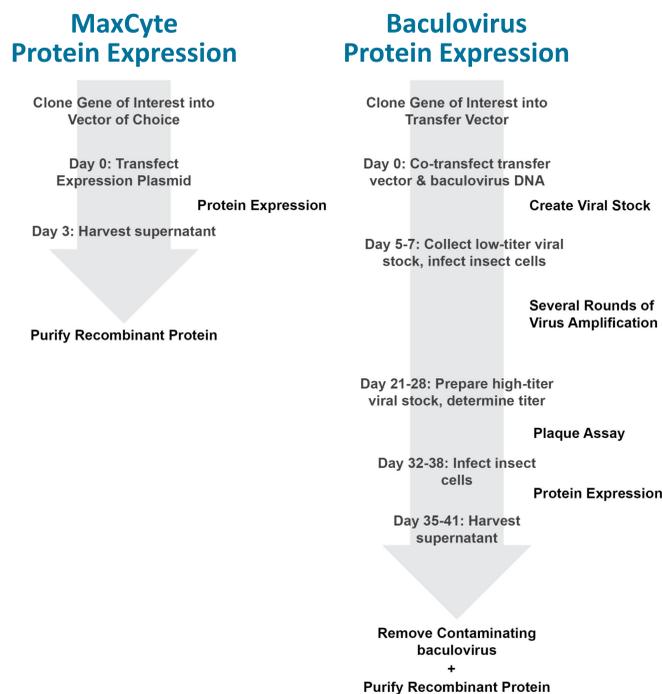
### High Efficiency Electroporation of SL3 Cells

Simple Assay Development: [DNA] vs. Expression Level



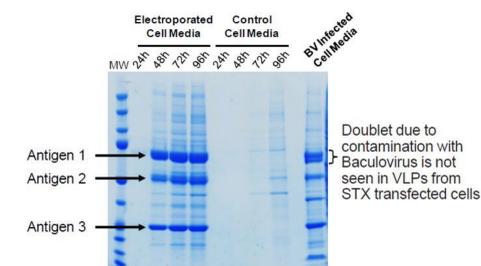
**Figure 3. High Efficiency Transfection of SL3 Insect Cells.** SL3 cells were transfected with 0, 1, or 2 µg/1E6 cells of a pGFP expression vector using the MaxCyte STX® Scalable Transfection System. Cells were examined for GFP expression using microscopy and FACS analysis 24 hrs post electroporation. Cell viability was determined by propidium iodide exclusion. Increased DNA concentration (2 µg/1E6 cells) lead to higher mean fluorescent intensity demonstrating the ability to control expression levels by altering DNA concentrations.

## MaxCyte Electroporation vs. Baculovirus Protein Production



## VLP Production from Sf9 Insect Cells

MaxCyte Transfection Eliminates the Potential for Baculovirus Contamination

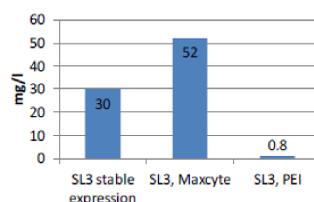


**Figure 4. Sf9 VLP Production Using MaxCyte Electroporation.** Sf9 cells were transfected with a single plasmid encoding three antigens that co-assemble into VLPs. Culture media was collected at various times from cells post electroporation or following baculovirus infection and analyzed using SDS PAGE.

## MaxCyte Electroporation vs. PEI

### Increased Expression in SL3 Cells

MaxCyte Transfection: Better 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.

## Summary

- MaxCyte electroporation enables rapid transient (co)transfection producing high cell viabilities & transfection efficiencies for a variety of insect cell lines.
- The level of protein expression can be controlled via [DNA].
- MaxCyte electroporation enables progression from gene to protein production in as few as 3 days.
- MaxCyte electroporation eliminates the need to use baculovirus expression systems, thus greatly shortening the protein expression timeline.
- Direct transfection via electroporation eliminates the potential for baculovirus particle contamination, simplifying and improving protein purification.
- MaxCyte electroporation outperforms chemical transfection methods such as PEI enabling high-yield protein production.
- MaxCyte insect cell transfection can produce a variety of proteins from simple secreted proteins to more complex products such as VLPs.

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