

Rapid and Scalable Transient Transfection Technology for High Titer Protein Production in HEK, CHO and Other Cell Types.

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MaxCyte STX™
SCALABLE TRANSFECTION SYSTEM



Abstract

Many drug discovery activities rely on production of pre-clinical quantities of recombinant proteins. Transient transfection offers a means of rapidly expressing a variety of proteins including antibodies, 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 and cell type flexibility. Flow electroporation transient transfection can produce proteins faster than stable cell lines, from a variety of adherent and suspension cell types. In this poster we describe a method for high quantity protein production based on scalable electroporation using the MaxCyte STX Transfection System. Data will be presented for high efficiency transfection of cells commonly used in protein production including CHO, HEK293 and insect cells using both small and large scale electroporation formats. Results from comparisons to other transient transfection technologies such as lipid reagents and PEI will show the superior utility and quality of MaxCyte electroporation.

MaxCyte Transfection



- Simple Operation & Assay Optimization
- Rapid
- High efficiency
- Broad cell type compatibility
- Scalable

MaxCyte® STX™ Scalable Transfection System. The MaxCyte STX system provides a rapid, scalable method for transiently transfecting a variety of cell types with DNA, RNA, siRNA, proteins or other biomolecules of interest. The MaxCyte STX can reproducibly express proteins in a variety of adherent and suspension cell types commonly used for protein production in the quantities required for most pre-clinical drug development activities.

The MaxCyte STX comes pre-loaded with specialized electroporation (EP) protocols for individual cell types. Standard MaxCyte protocols provide an optimal blend of loading efficiency and viability, which are ideally suited for generating cells for use in cell-based assays. MaxCyte has developed additional CHO and HEK EP protocols designed specifically for high level protein expression. Identical protocols are used for transfecting cells in either small scale (5×10^5 to 4×10^7 cells in seconds) or in large scale (up to 1×10^{10} cells in less than thirty minutes). After identifying a DNA concentration that yields optimal assay results at small scale, the EP process can be scaled up without impacting transfection efficiency or cell viability for use in full-scale, protein production.

Insect Cell Transfection

SL3 Cells GFP Transfection

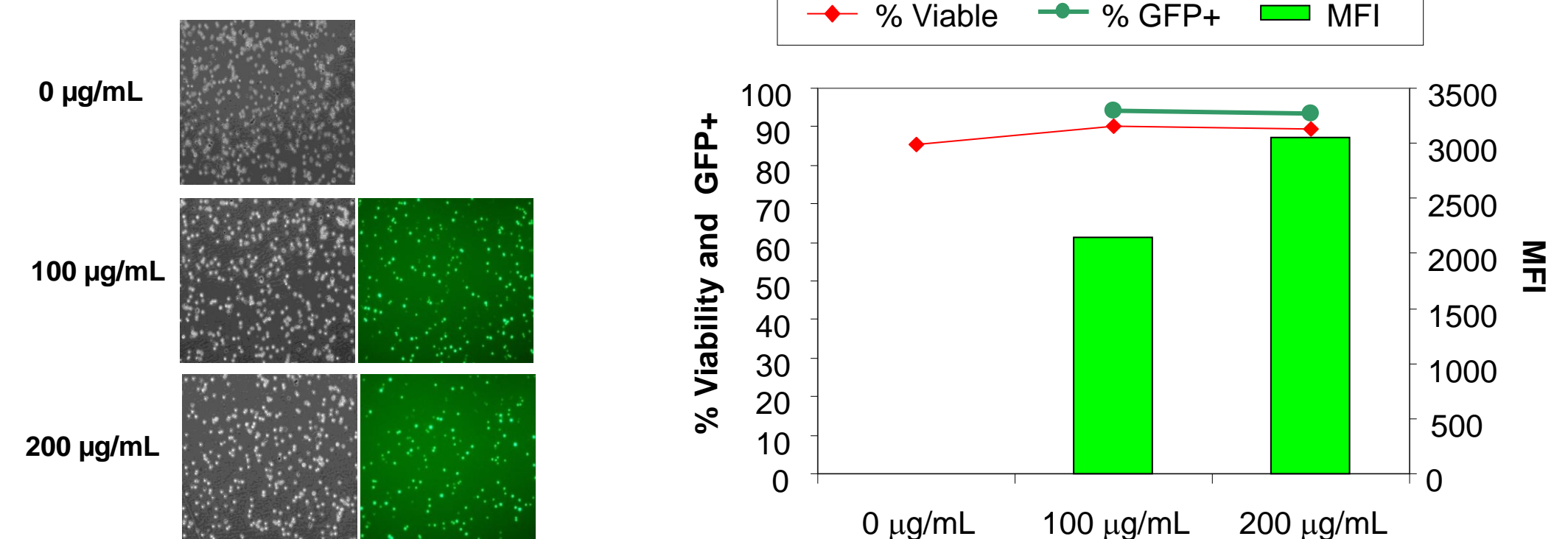


Figure 1. Protein Expression in Insect Cells. SL3 cells were transfected with varying concentrations of pLEX4GFP. Cell viability and GFP expression were assessed 1 day post EP using microscopy and FACS analysis. Cell viability was unaffected by EP. Loading efficiencies exceeded 90% with both sets of cells. Mean fluorescence intensity (MFI) correlated with DNA concentration, illustrating how MaxCyte STX users can control transgene expression levels by varying loading agent concentrations. SF9 insect cells have also been successfully transfected with this same expression plasmid (data not shown).

Viral Protein & Vector Production: HEK Cells

Transfection Scalability: Lentivector Production using HEK 293FT Suspension Cells

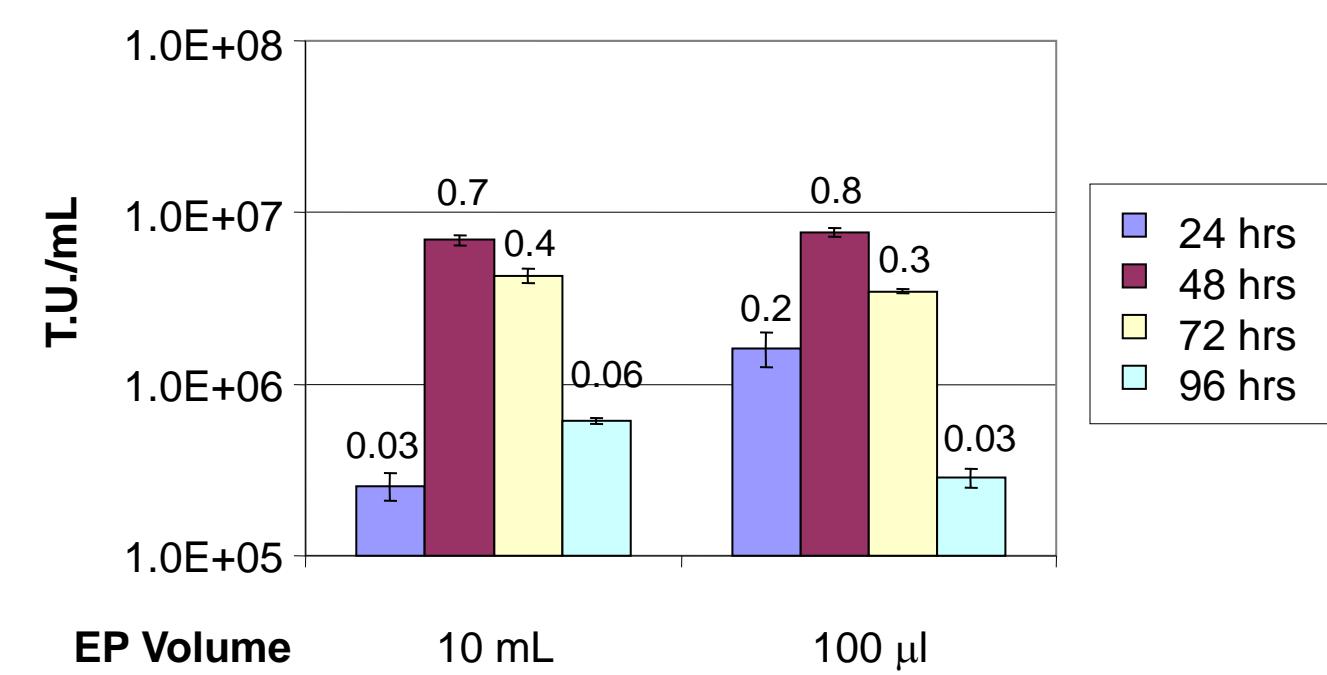


Figure 2. Small and Large Scale Lentiviral Vector Production in HEK Suspension Cells. Suspension-adapted 293FT cells were expanded in T175 shake flasks and transfected in 10 mL and 100 µl EP reactions with equivalent concentrations of a 4 plasmid mixture encoding the components of an HIV-based lentiviral vector system with a GFP transgene. (plasmids courtesy of Dr. Ken Cornetta, Indiana University School of Medicine). Virus was collected every 24 hours for 4 days and titrated by transducing 293T cells. These data highlight the ability to perform assay optimization using small scale electroporation and migrate to large scale (flow) electroporation without the need for further assay development. They also illustrate how the MaxCyte STX enables high titer viral vector production with suspension-adapted cells, which are better suited to large scale manufacturing relative to adherent cells.

Large Scale Transfection: Lentivector Production using HEK 293FT Adherent Cells

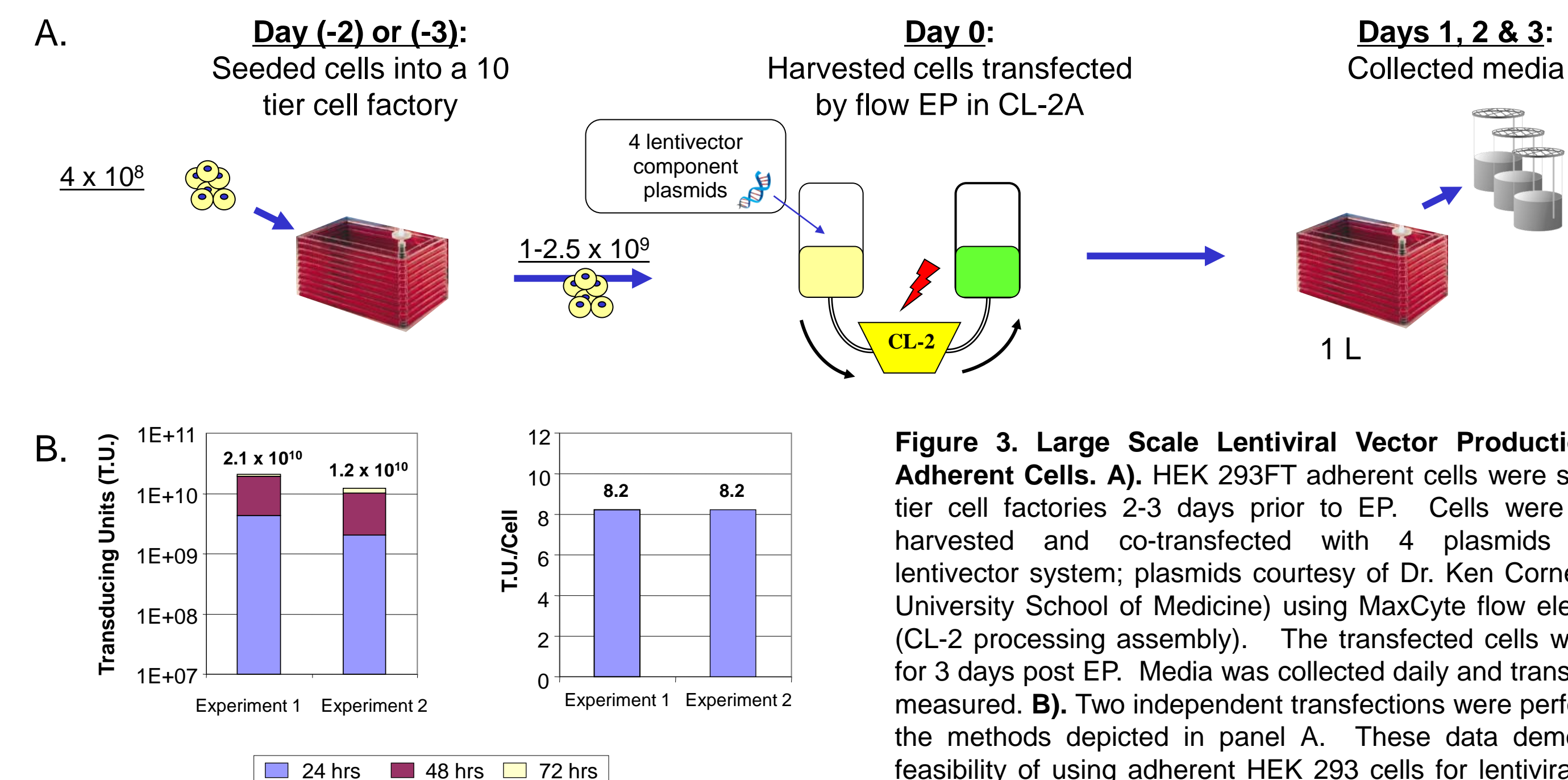


Figure 3. Large Scale Lentiviral Vector Production in HEK Adherent Cells. **A).** HEK 293FT adherent cells were seeded in 10 tier cell factories 2-3 days prior to EP. Cells were trypsinized, harvested and co-transfected with 4 plasmids (HIV-based lentivector system; plasmids courtesy of Dr. Ken Cornetta, Indiana University School of Medicine) using MaxCyte flow electroporation (CL-2 processing assembly). The transfected cells were cultured for 3 days post EP. Media was collected daily and transducing units measured. **B).** Two independent transfections were performed using the methods depicted in panel A. These data demonstrate the feasibility of using adherent HEK 293 cells for lentiviral production as well as the high level of transfection reproducibility.

Viral Protein Production in HEK 293F Cells

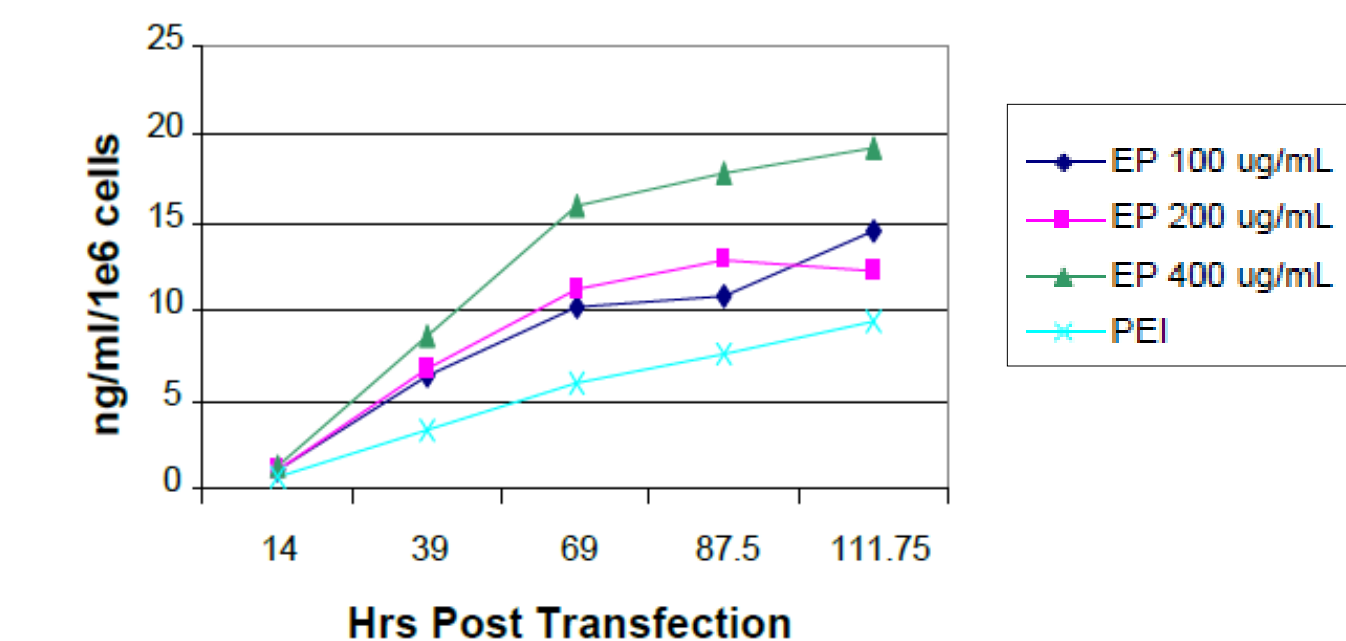
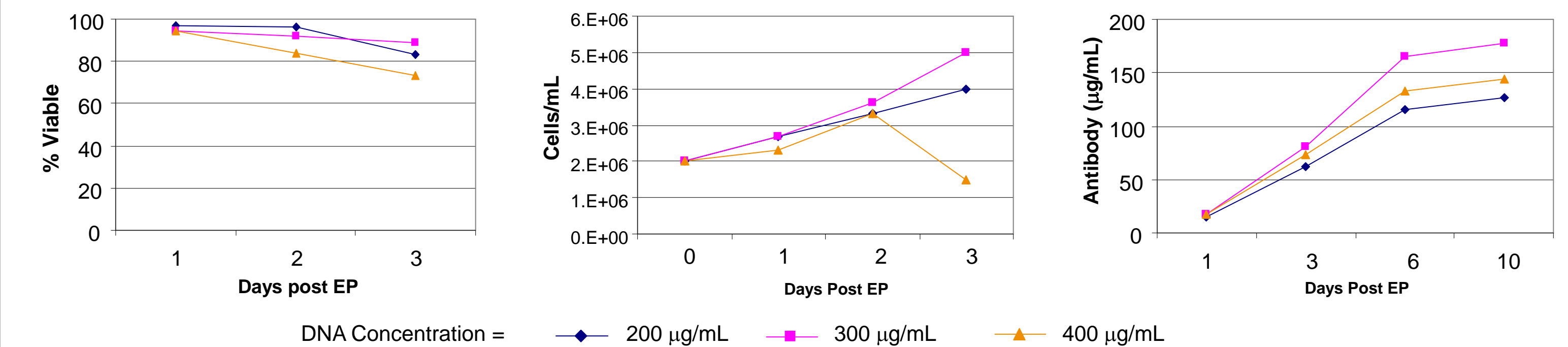


Figure 4. Recombinant Viral Protein Production via Electroporation or PEI. HEK 293F cells were transfected with varying concentrations of a viral protein expression plasmid using MaxCyte STX electroporation or via an optimized polyethyleneimine (PEI) method. Cells were cultured for approximately 5 days. Culture media was collected at various times post transfection and protein titers measured via ELISA.

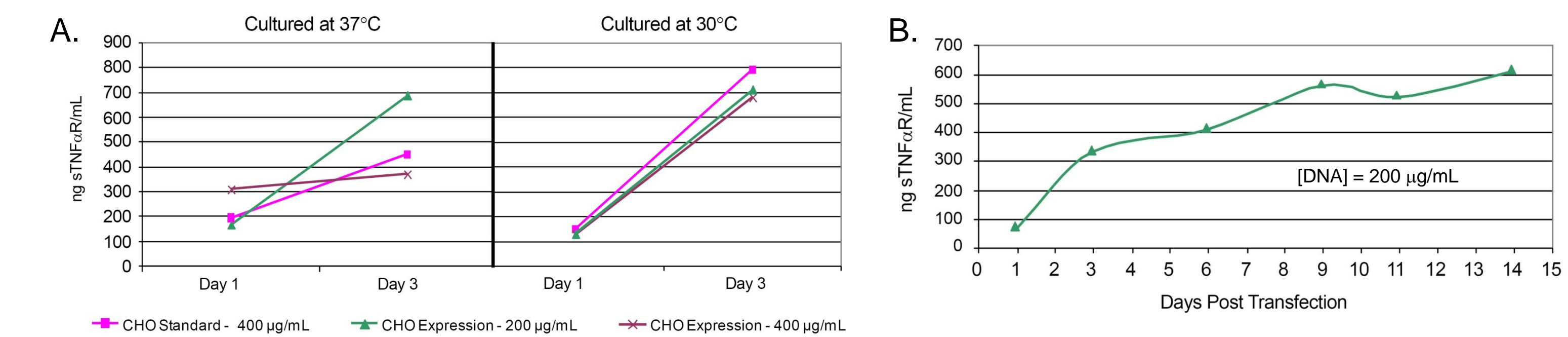
Antibody Production: CHO Cells

Antibody Production in CHO Cells



Secreted Protein Production: CHO Cells

TNFαR Production Method Development



Summary

- MaxCyte EP can be used to express a variety of proteins including antibodies, secreted proteins, viral vectors and recombinant viral proteins in quantities commonly needed for pre-clinical studies.
- MaxCyte EP can be used to (co)transfect a variety of cell types such as insect cells, adherent cells and suspension cell lines, including CHO and HEK cells. MaxCyte scientist have developed EP protocols which produce high levels of transfection efficiency and cell viability and maximum protein expression.
- The MaxCyte STX transfection system is fully scalable. Researchers can rapidly develop a protein production method using small scale EP, followed by seamless migration to large scale protein production without further optimization.
- MaxCyte EP is compatible with cell types commonly used for clinical-scale protein production via stable cell lines allowing for overlap between rapid pre-clinical production and full scale clinical protein production.

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