

Rapid Development of a High Titer Protein Production Method with CHO Cells Transiently Transfected using MaxCyte Electroporation Technology

Introduction

Production of proteins is needed for a variety of drug discovery activities including clinical trials, biotherapeutic antibody and recombinant protein manufacturing, vaccine, viral vector and virus-like particle (VLPs) production as well as purified proteins for use in biochemical screening and profiling. Currently, there is a push to make go/no go decisions earlier in product development and thus an increased demand for larger amounts of the protein of interest earlier in drug discovery programs. Stable cell lines have been the standard for protein production for over two decades. Transient transfection, however, has become a practical solution to the time, labor and cost challenges faced when relying exclusively on stable cell lines. Both stable cell lines and transient transfection have important roles during different stages of drug discovery.

Transient transfection offers a means of rapidly expressing proteins, although not all transient transfection technologies meet the requirements for protein production, namely scalability, cell type flexibility and cost-effectiveness. The MaxCyte[®] STX[™] Scalable Transfection System reproducibly transfects a variety of adherent and suspension cell types enabling production of proteins in the quantities needed for most pre-clinical product development. Ideally, researchers would use the same cell system for both pre-clinical and clinical stage product development. Thus it makes sense to retain CHO cells as the production vehicle during transient transfection. The MaxCyte STX provides a rapid, scalable method for transient transfection of primary cells, cell lines and importantly, historically difficult to transfect cell lines such as CHO cells.

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 cell viability, which are ideally suited for generating cells for use in cell-based assays. MaxCyte has developed additional EP protocols for CHO and HEK cells that are designed specifically for high level protein expression. These protocols are used for transfecting cells both in small scale (5×10^5 to 4×10^7 cells in seconds) and large scale formats (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.

In this application note we review the rapid development of a high titer protein expression system in CHO suspension cells using MaxCyte's proprietary scalable electroporation technology. We demonstrate how MaxCyte's optimized protein expression protocols provide the ability to load cells with greater quantities of DNA relative to the standard CHO protocol. As a result, average protein expression per cell is increased. In addition, we illustrate the effects of cell culture conditions on protein production in transiently transfected cells. Finally, we show that titers of soluble TNF α R increase for at least one week and are sustained for up to two weeks following electroporation of CHO cells with a sTNF α R expression plasmid.

Materials

CHOs cells (Invitrogen 11619012)
CD-CHO (Invitrogen 10743-029)
Penicillin-streptomycin (Mediatech 30-002-CI)
HT Supplement (11067030)
Qiagen (Giga Kit for plasmid preparation 12991)
R&D System (sTNF α Receptor Detection, DRT 200)

Methods

Cell Culture and Electroporation:

Suspension-adapted CHO cells were cultured at 37°C with 5% CO₂ in CD-CHO media containing 1% HT Supplement and 1% pen/strep. Cells were passaged every 2-3 days, and split one day prior to EP to ensure they were in log phase growth at the time of transfection.

Cells were pelleted at 250 X g for 10 minutes, rinsed in MaxCyte EP buffer (2-5X final electroporation volume), and suspended in MaxCyte EP buffer at 1×10^8 cells/mL. Cells were mixed with plasmid DNA and transferred to OC-100 or OC-400 processing assemblies. Cells were EP'd using either the preset "CHO" or "CHO Protein Expression" protocol and immediately transferred to one well of a 96-well (OC-100) or 6-well (OC-400) plate. After a 20 minute incubation at 37°C in a 5% CO₂ incubator, the cells were diluted in cell culture medium at a density of 5×10^5 or 1×10^6 cells/mL and cultured in T25 shake flasks.

FACS Analysis:

Cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson). Viability was assessed by the addition of 0.625 $\mu\text{g}/\text{mL}$ propidium iodide.

TNF α R Quantification:

Conditioned media samples were removed without replacement and stored at -80°C . Protein titers were measured by ELISA using manufacturer's (R&D Systems) standard protocol.

Results

Figure 1 illustrates the relative effects of electroporation energy and DNA concentration on cell viability and transgene expression in CHO cells. Increased electroporation energy resulted in the ability to load CHO cells with greater quantities of pGFP DNA and in turn, lead to an increase in average GFP expression per cell when compared with the standard CHO protocol. Note that in the absence of DNA, electroporation had little impact on viability, even when using the higher energy protocol. However, the average cell viability was reduced due to DNA toxicity, and correlated directly with the amount of DNA loaded into the cell.

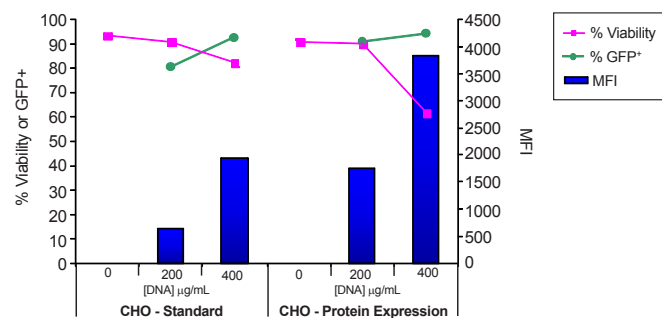


Figure 1: Increased Cell Loading with Optimized Electroporation Parameters. CHO cells were electroporated (EP) with 0, 200 or 400 $\mu\text{g}/\text{mL}$ pGFP in small scale format (OC-100 processing assemblies) using either MaxCyte's standard CHO protocol or a CHO-specific protocol optimized for protein expression. Cells were seeded in shake flasks at approximately 1×10^6 cells/mL and assayed by FACS ~20 hrs post electroporation. Data are expressed for % cell viability, % GFP $^{+}$ cells and the mean fluorescence intensity (MFI).

In Figure 2, CHO cells were transfected with varying concentrations of an expression plasmid encoding soluble TNF α receptor using the standard or expression-optimized EP protocols. The secreted TNF α R protein titers on Day 1 post EP reflect the relative protein expression levels that were observed for the GFP experiment in Figure 1.

After culturing TNF α R transfected cell populations for 3 days at 37°C , the effects of DNA toxicity at 400 $\mu\text{g}/\text{mL}$ were manifested as lower relative TNF α R titers. The effects of DNA toxicity were mitigated by culturing the transfected cells at 30°C instead of 37°C . TNF α R titers in all three cell populations were higher after three days in culture when incubated at 30°C than in the parallel 37°C cultures.

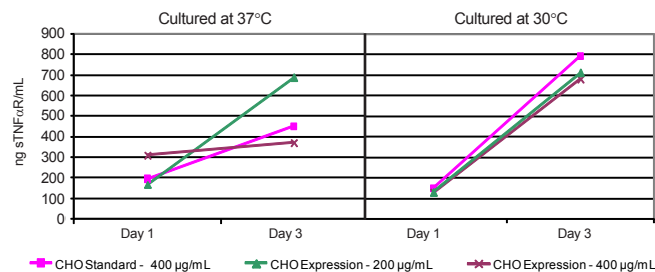


Figure 2: Post Electroporation Culture Temperature Optimization. Cells were EP'd with pTNF α R expression plasmid in OC-400 processing assemblies using either the standard or expression-optimized CHO protocol. Cells were seeded into duplicate shake flasks at 1×10^6 cells/mL, and cultured at 37°C or 30°C . Conditioned media samples were collected without replacement at days 1 and 3 post EP and assayed for TNF α R production by ELISA.

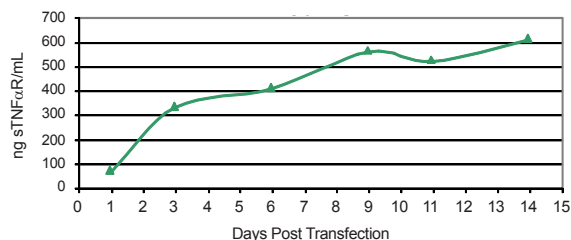


Figure 3: Sustained sTNF α R Production at 30°C . Cells were EP'd with 200 $\mu\text{g}/\text{mL}$ pTNF α R plasmid using the CHO expression-optimized protocol, seeded into shake flasks at 5×10^5 cells/mL and cultured at 30°C . Conditioned media samples were collected daily for 15 days post EP and TNF α R quantified.

To examine the duration of protein expression, cells transfected with the TNF α R plasmid were cultured at 30°C for over two weeks. Although the MaxCyte STX system transiently transfects cells, TNF α R protein titers increased for up to 2 weeks post EP. The duration of protein production will depend on factors such as protein half life, media composition, cell type and vector design, but these data strongly suggest that transient transfection using the MaxCyte STX is a viable alternative to stable cell lines for pre-clinical protein production.

Protein Production Recommendations

- To optimize conditions for high titer production of a particular protein in CHO and HEK cells, STX users should conduct several small scale electroporations with varying concentrations of their expression plasmid using both the standard and protein expression protocols. DNA concentrations in the range of 200-400 $\mu\text{g}/\text{mL}$ are a good starting point. In some cases, lower or higher concentrations may yield better titers, depending on the relative toxicity of the expressed protein, strength of the promoter and physiology of the cell line.
- In addition to DNA concentration and electroporation energy, cell culture conditions after electroporation have a significant impact on protein titers. Factors such as media composition, cell seeding density, culture temperature and media collection schedules will influence the amount of protein that can be generated by transfected cells.