Large-Scale Cell Concentration and Buffer Exchange Prepares CHO Cells for Electroporation

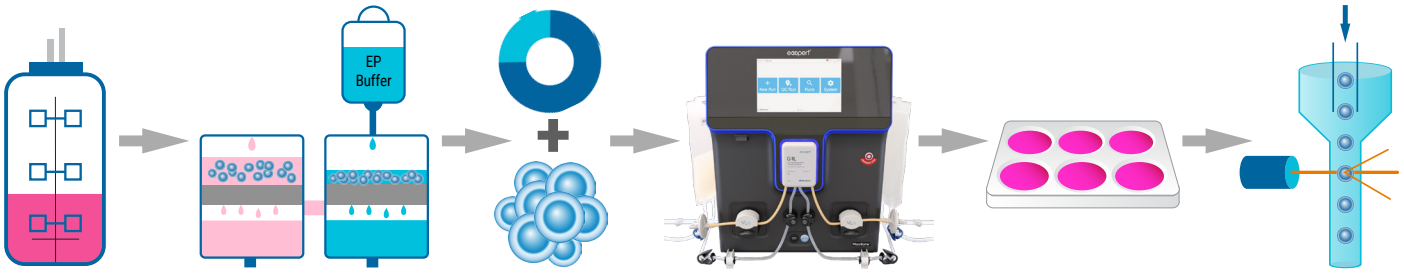
Scientific Brief

Abstract

Recombinant protein production is an essential step in the development of many novel, life-saving therapies and diagnostics. Stable cell line development enables consistent protein yields and product quality, but the process is lengthy, costly and time-consuming. Transient protein expression could be a viable alternative to stable cell line development producing material for pre-clinical development and early clinical trials.

To realize the potential of transiently expressed protein, very large quantities of transfected cells are required. MaxCyte's ExPERT VLx™ enables the transfection of up to 200 billion cells in under 30 minutes. Cell concentration and buffer exchange prepare cells for electroporation; one solution is tangential flow filtration (TFF), which offers gentle processing that is scalable, fast and easy to use. In collaboration with Repligen, we developed an optimized process using the KTF-600 KrosFlo® system and hollow fiber TFF devices to deliver CHO cells in MaxCyte electroporation buffer, at the required concentration of $2x10^8$ cells/mL.

Experimental Design



Seed Train

CHO Seed Train (20L) in a bioreactor

Cell Concentration and Buffer Exchange

Cells were concentrated to 700 mL in MaxCyte EP buffer by Repligen TFF

Electroporation

1 mL cell aliquot was transfected with plasmid DNA

Resting

Transfected cells rested and were cultured for 24 hours

Analysis

Transfection efficiency was assessed by Flow Cytometry

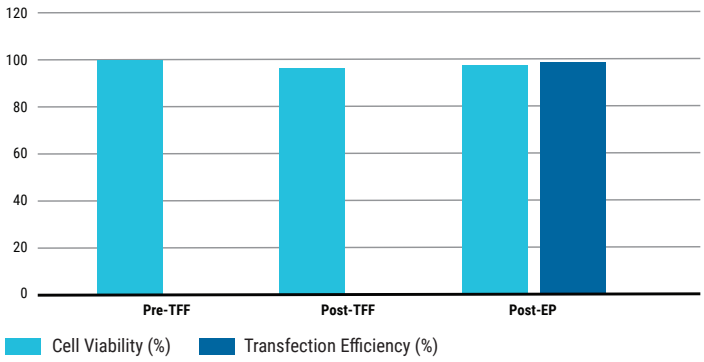


Figure 1. Cell concentration and buffer exchange yields healthy, electroporation-ready cells which can be efficiently transfected. CHO cells from the seed train at $8x10^6$ cells/mL in growth media were concentrated to $2x10^8$ cells/mL in MaxCyte electroporation buffer. Vi-CELL™ analysis confirmed that high cell viability was maintained throughout. Cells were transfected with 300µg/mL of pGFP DNA using the MaxCyte VLx. Transfection efficiency was assessed 24 hours post electroporation; 99% of transfected cells expressed GFP.

Summary

- This proof-of-concept study confirms that Repligen's KTF-600 KrosFlo® and hollow fiber TFF devices provide a gentle, efficient method for large-volume cell concentration and buffer exchange prior to electroporation.
- The concentrated cell volume achieved enables electroporation of up to 200 billion cells, in a single run on the MaxCyte VLx , for high-efficiency transfection in under 30 minutes.
- Repligen's KTF-600 KrosFlo system can be connected to the MaxCyte VLx in a sterile manner for a fully closed process.

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