Laser-Enabled Analysis and Processing Data Supports Superior Performance of Animal Component Free Electroporation and Recovery Formulation

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Introduction

Electroporation is widely used for gene delivery in cell line generation, and is the method of choice for serum-free suspension culture transfection. Electroporation protocols and media can have a major impact on post-electroporation cell viability, recovery cell density, and transfection efficiency.

SAFC Bioscience's animal component free (ACF) electroporation and recovery formulation was previously shown to achieve equivalent or higher post-electroporation viabilities as well as equivalent or higher viable cell densities after 24 hours recovery compared to other media tested. These results were confirmed. Using a fluorescence platereader to measure transfection level, this formulation was also shown to facilitate higher transient protein expression levels than other media. Recently, we were able to more accurately measure transfection levels in different media conditions using Laser Enabled Analysis and Processing (LEAP).

LEAP is a technology that can be utilized for a number of purposes including transfection of macromolecules, assessment of cell viability and growth, assessment of expression of secreted and non-secreted proteins by individual cells, and targeting and destruction of selected cells in a population. For our purposes, the LEAP instrument was used in two ways. First, we used it to calculate the number of cells expressing transfected plasmid DNA compared to the total number of cells present for each test condition. This value was labeled transfection efficiency. Second, we used it to quantify the level of Green Fluorescent Protein (GFP) expression per transfected cell in the samples analyzed. Our results indicated that cells electroporated and recovered in our optimized formulation have an equivalent transfection efficiency compared to competitor media tested. However, cells transfected and recovered in our formulation exhibit a population shift that reflects higher levels of recombinant protein expression per transfected cell.

Material and Methods

Cell Line and Media:
The stock cultures of parental CHO K1 cell line (ATCC) were maintained in suspension culture in EX-CELL™ CHO DHFR- Medium (SAFC Biosciences **Catalog No. C8862**). All SAFC Biosciences formulations were supplemented with 4 mM L-Glutamine. Competitor Medium A was supplemented with 8mM L-Gln and 1X HT Supplement according to manufacturer's instructions. Competitor Medium B was used without additional supplementation.

Plasmid Electroporation
The parental CHO K1 cells were transfected with a proprietary GFP expression vector in the test media by electroporation, using a Gene Pulser II Electroporator (Bio-Rad). 4 mm-gap electroporation cuvettes (Bio-Rad and Sigma-Aldrich) were chilled on ice for 10 minutes prior to transfection.

Parental CHO K1 stock culture was centrifuged at 200 RCF for 5 minutes and re-suspended in test media at 5.0×10^6 viable cells/mL. 0.8 mL cell suspension was gently mixed with 50 µg of sterile plasmid DNA in a sterile Eppendorf tube and transferred to an electroporation cuvette. Electroporation was conducted in exponential decay mode (time constant ∞) at 300 Volts and 950 µF capacitance. The electroporated cell suspension was then immediately transferred into 5mL test medium for recovery. Samples were taken at this point to evaluate post-electroporation viability using a ViCell XR Automated Cell Viability Analyzer (Beckman-Coulter). The remaining suspension was then recovered for 24 hours at 37 °C, 5% CO_2 in T25 tissue culture flasks (Corning) coated with 500ul of 60mg/ml Poly (2-hydroxyethyl methacrylate) (Sigma) to inhibit cell attachment to the tissue culture flask surface.

Cell Recovery
Twenty-four hours post-electroporation, all cells from each T-25 flask were collected, centrifuged at 200 RCF for 5 minutes, and re-suspended in 1.3 mL Phosphate Buffered Saline (PBS). 550 mL of the cell suspension was used to perform viable cell density counts using ViCell. Viable cell densities from each test condition were normalized to the value of the C6366 test condition (not shown). Remaining suspension was used for LEAP analysis of GFP Expression.

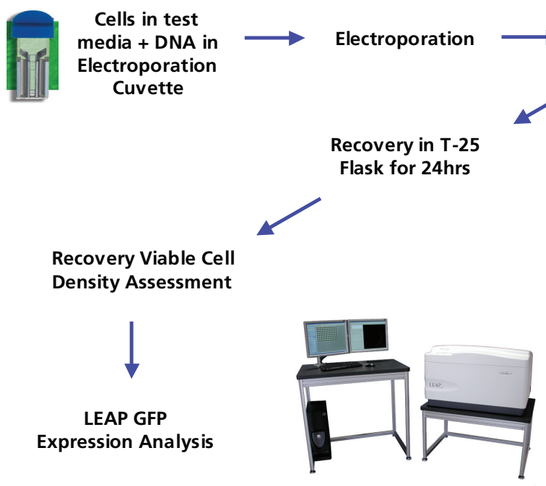


Figure 1: **Electroporation and Recovery Procedure.**

LEAP GFP Expression Analysis Procedures
Transfected or mock-treated cells were seeded at equivalent densities into 384-well C-Lect™ plates for visualization on the LEAP instrument.

Transfection Efficiency: Using both brightfield and fluorescent channel imaging, the total number of cells and the total number of GFP-expressing cells in each well were quantified via the Cell Counting program that is available on the LEAP technology platform. Quantified results from each well were then tabulated to determine the exact transfection efficiency for each experimental set by dividing the number of GFP-positive cells in each well by the total number of cells in each well.

Evaluation of GFP Expression Levels Per Cell: Total fluorescence intensity values for each transfected cell that was counted were categorized into Low (below 300,000 fluorescence units), Medium (from 300,000 to 600,000 fluorescence units), and High (above 600,000 fluorescence units) groups. The percentage of the total number of cells in each of these groups was determined for each media tested.

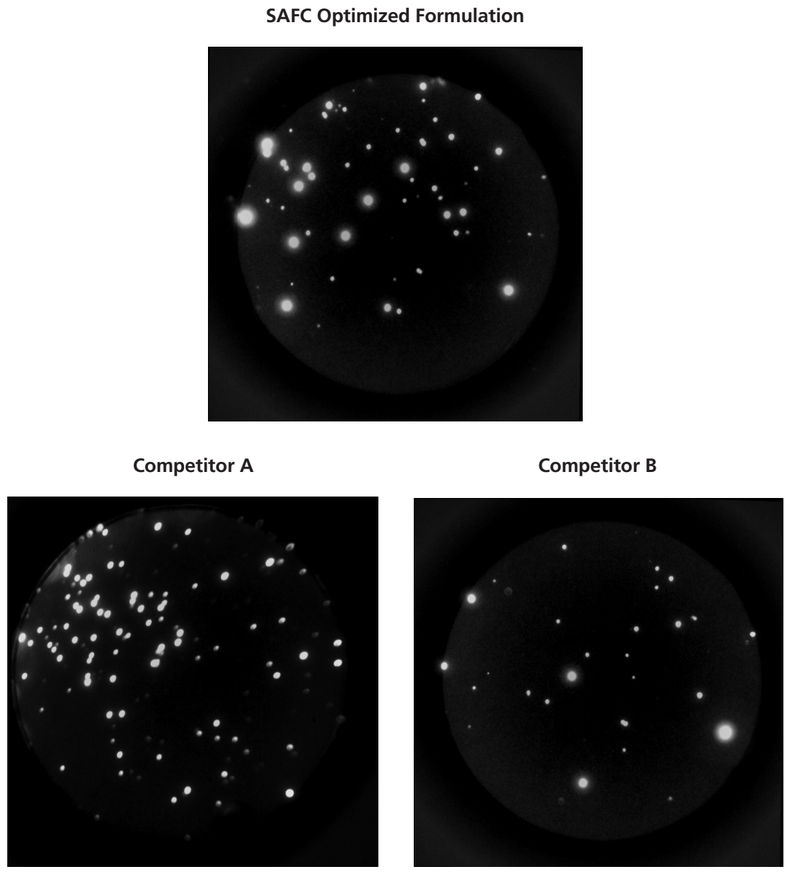
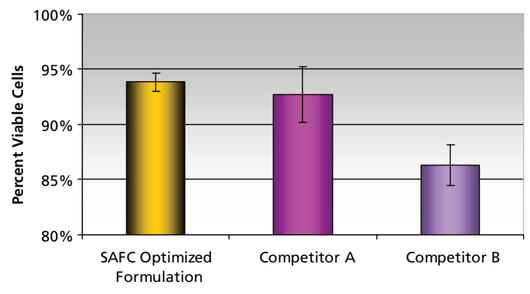
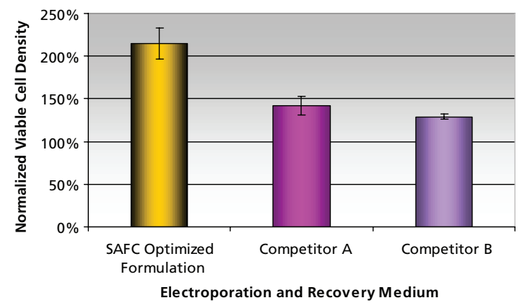


Figure 2: **LEAP Images of Transient GFP Expression.**

Results and Discussion



3A: Post-Electroporation Viability.



3B: Viable Cell Density after 24 Hours Recovery (Normalized to Positive Control Not Shown)

Figure 3: **Confirmatory Results.**
Previous conclusions are confirmed. SAFC's optimized ACF electroporation and recovery formulation achieves equivalent or higher post-electroporation viabilities **(Figure 3A)** as well as equivalent or higher viable cell densities after 24 hours recovery **(Figure 3B)** compared to other media tested.

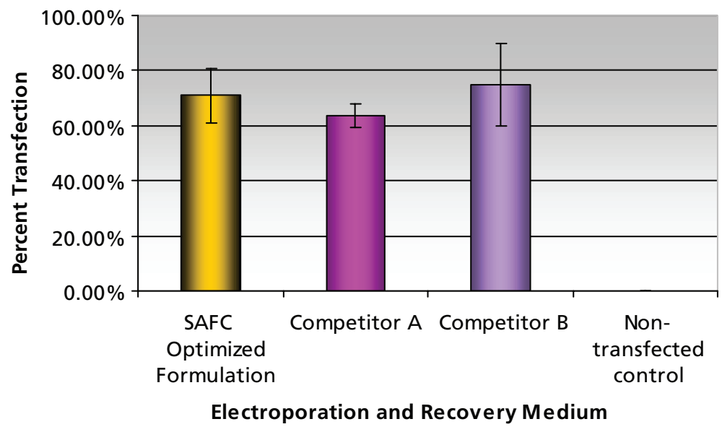


Figure 4: **Transfection Efficiency.**
Data generated by the LEAP instrument indicates that cells electroporated and recovered in SAFC's optimized formulation have transfection effieciencies similar to Competitor Medium A and Competitor Medium B **(Figure 4)**. The non-transfected control indicates no background fluorescence detected by the LEAP instrument.

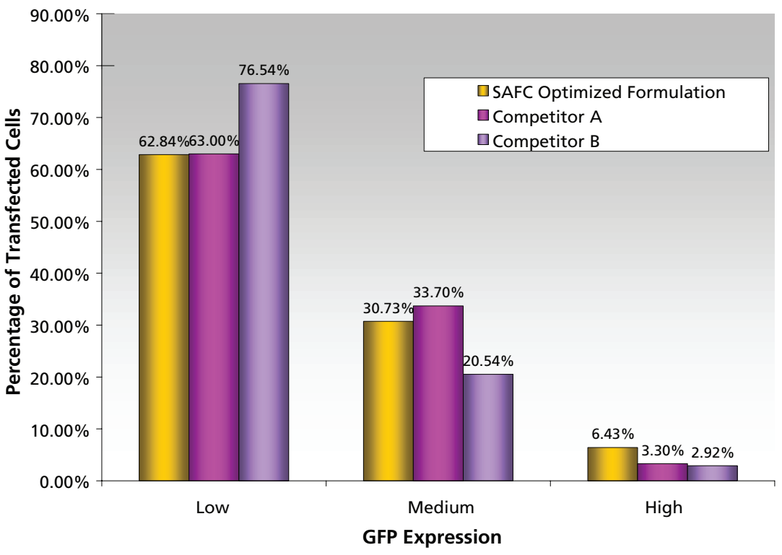


Figure 5: **Transient GFP Expression.**
Figure 5 demonstrates that cells transfected and recovered in the SAFC optimized formulation exhibit a population shift that reflects higher levels of recombinant protein expression per cell when compared to Competitor Medium A or Competitor Medium B. The percentage of the population of cells transfected and recovered in the SAFC optimized formulation shows a greater proportion of individual cells producing high levels of recombinant GFP.

Conclusions

- Electroporation and recovery in SAFC Bioscience's optimized ACF formulation result in equivalent or higher post-electroporation viabilities and viable cell densities after 24 hours recovery compared to other media tested.
- The LEAP instrument can be used to determine transfection efficiency and quantify the expression level of fluorescently labeled protein(s) in individual cells.
- Transfection efficiencies are equivalent whether cells are electroporated and recovered in the SAFC optimized formulation, Competitor Medium A, or Competitor Medium B.
- Cells transfected and recovered in the SAFC optimized formulation exhibit a population shift towards higher levels of recombinant protein expression compared to Competitor Medium A or B. This observation supports previous RFU data obtained from fluorescence platereader measurements.