Mirus

Electroporation Made Easy for Hard-to-transfect Cells

Mirus Bio Ingenio® EZporator® Electroporation System makes gene delivery simple with high efficiency electroporation of diverse cell types, including primary human T cells

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ABSTRACT

Electroporation is a highly effective, versatile technique for the delivery of molecular cargo to many different cell types and tissues of biomedical relevance. Despite its efficacy, electroporation is under-utilized because of the perceived initial up-front cost in purchasing an electroporator and its association with high cytotoxicity. Another deterrent to using this method is that some widely used electroporation systems only allow users to select from non-adjustable, pre-defined programs which obfuscate basic parameters, e.g. voltage, of the electrical pulse to be delivered. Here, we dispel the misconception that electroporation is necessarily fraught with high cytotoxicity and complexity by introducing the cost-effective Ingenio® EZporator® Electroporation System, which incorporates 'what you see, is what you get' design principles. By simply adjusting the sole input (voltage) of the Ingenio® EZporator®, gene delivery efficiency and cellular viability can be easily optimized for cell types which are notoriously difficult to transfect with chemical transfection reagents.

INTRODUCTION

Among the trifecta of commonly used approaches for gene delivery (electroporation, chemical transfection, and viral transduction), electroporation is distinct in that it delivers naked nucleic acids and does not require complexation of cargo molecules with chemicals or packaging into a viral vector (**Table 1**). Instead, cargo molecules cross the lipid bilayer of cells through pores which are formed through application of an electrical field.[1,2] As such, the efficacy of electroporation hinges on the physical parameter of transmembrane voltage instead of the nuanced endocytic mechanisms that can limit transfection and transduction efficiency. So, for "hard-to-transfect" cell types which are resistant to chemical- or virus-mediated methods of gene delivery, electroporation is a valuable alternative.

Though puncturing cell membranes with electric pulses may sound rather brutish, pore formation is reversible, and cell survivability is relatively high under optimized electroporation conditions. Electroporation conditions with the Mirus Bio Ingenio® EZporator® System can be easily adjusted to balance transfection efficiency with cellular viability. In this white paper, this elegantly simple system for electroporating diverse cell types with biologically relevant cargos is discussed. With one universal electroporation buffer, the Ingenio® EZporator® Electroporation System is capable of electroporating cells which have immense biomedical and biotechnological potential, including those that are notoriously difficult to transfect with commercially available chemical transfection reagents such as primary T cells.

TABLE 1. Features of common gene delivery techniques.

	Electroporation	Chemical Transfection	Viral Transduction
Related Materials	electroporator, cuvettes, electroporation buffer	chemical reagents, e.g. *Trans*IT® reagents, calcium phosphate, cationic lipids and polymers, nanoparticles	viral vector-producing or packaging cell line
Mechanism of Action	permeabilization of cell membrane via applied electrical field	condensation and complexation of cargo, mediation of charge interactions between cargo and cell surface, endocytosis	depends on viral vector, entry of packaged cargo via viral infection of cells
Process	suspend cells in buffer and apply electrical pulse	add transfection complex mixture to cells	transfect cells to produce viral vectors, harvest and purify vectors, infect cells
Time Required	minimal	minimal	several days
Primary Cost	up-front purchase of electroporator	chemical reagents	time, chemical reagents
Primary Benefit	delivery of diverse cargo to hard-to-transfect cell types	convenience	efficient, targeted delivery to both *in vitro* and *in vivo* systems, including quiescent cells

INTRODUCING THE INGENIO® EZporator® ELECTROPORATION SYSTEM

The Ingenio® EZporator® is as easy to use as it looks (**Figure 1**). Measuring in at 10 inches (254 mm) at its widest dimension, the EZporator® pulse generator leaves a small footprint. This lightweight electroporator delivers exponential decay pulses at a user-determined voltage, i.e. the charge is released from the capacitor and voltage decays exponentially over time from a set value. Internal resistance and capacitance are fixed constants at 150 Ω and 1,050 µF / 36 µF (low / high voltage modes), respectively. For most applications in mammalian and insect cell culture, the low voltage mode, which ranges from 20-400 V with 2 V resolution, is recommended. The EZporator® Pulse Generator can also reach up to 2,500 V with 10 V resolution in the high voltage mode, which is appropriate for bacterial and yeast transformation, though electroporation of these microorganisms has not been tested by Mirus Bio.

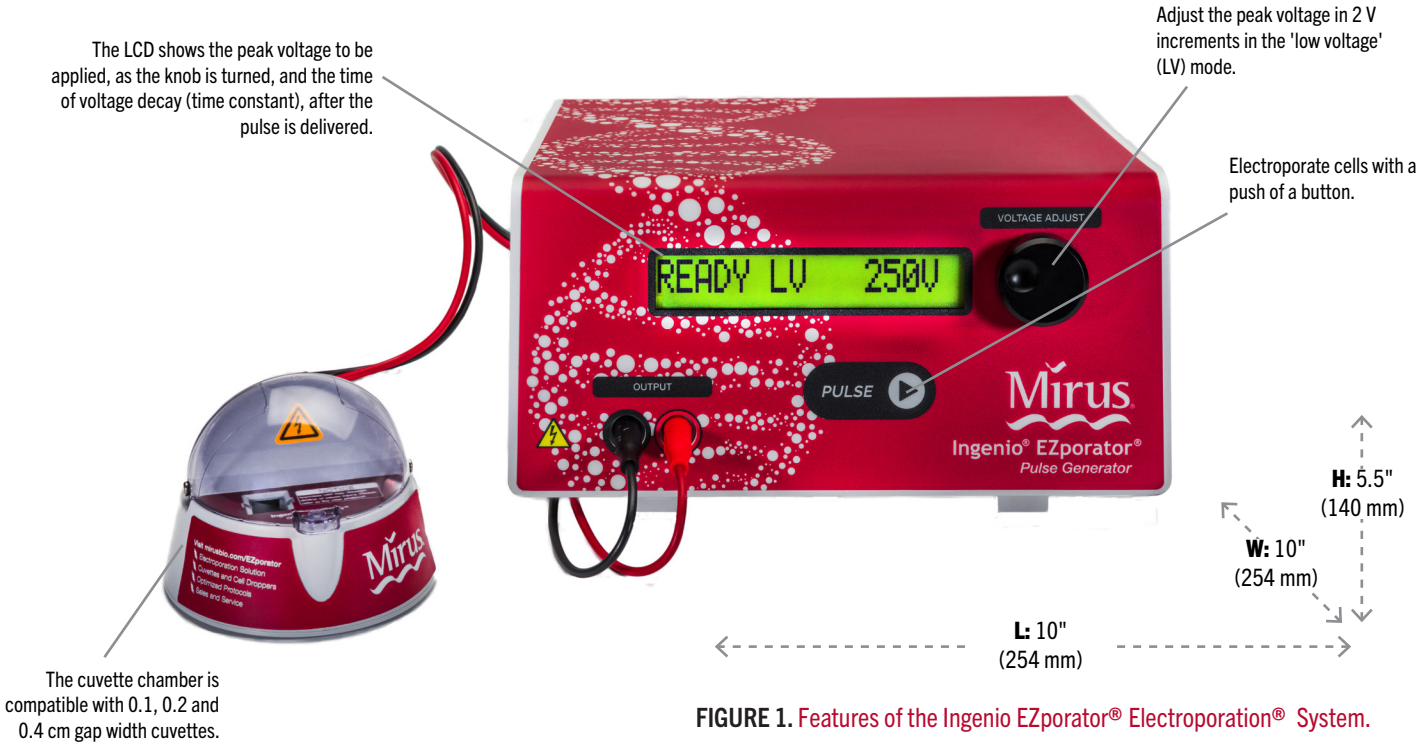


FIGURE 1. Features of the Ingenio EZporator® Electroporation® System.

INGENIO® ELECTROPORATION SOLUTION AND KITS

The Ingenio® Electroporation Solution (**Figure 2**) is a universal, high efficiency, low toxicity buffer for electroporating nucleic acids or other cargo molecules into mammalian and insect cell types. It is compatible with most commercially available electroporators and is a cost-effective alternative that generally performs equivalently to kits marketed for specific cell types. The EZporator® was designed for use with the Ingenio® Electroporation Solution and Kits.

FIGURE 2. Ingenio® Electroporation Kit.

OPTIMIZE WITH EASE

The electroporation process for the Ingenio® EZporator® Electroporation System is straightforward (**Figure 3**). For cells resuspended in Ingenio® Electroporation Solution, the optimal exponential decay pulse for most mammalian cell types falls within a range of 80-160 V, when using 0.2 cm cuvettes (100 µl volume) and 200-300 V, when using 0.4 cm cuvettes (250 µL volume). To determine the optimal voltage setting for a given cell type, a simple voltage titration can be performed.

Primary human T cells are difficult to transfect with non-viral methods. To determine optimal electroporation conditions for plasmid delivery to this difficult-to-transfect cell type, we performed a voltage titration to identify settings that would result in the highest viability and greatest number of cells expressing a transgene using the Ingenio® EZporator® Electroporation System (**Figure 4**). Three days prior to electroporation, freshly harvested T cells were activated with CD3/28 in IL-2 conditioned medium. The cells (10×10^6 cells/ml) were electroporated in 0.2 cm cuvettes with an eGFP-reporter plasmid (20 µg/ml). The entire electroporation volume of 100 µl was plated in one well of a 12-well plate and assessed three days post-electroporation. GFP expression, cell count and viability using propidium iodide were determined by flow cytometry. The results of this experiment suggest a voltage setting of ~170 V (0.2 cm cuvettes) is optimal for electroporating primary human T cells with the Ingenio® EZporator® and Electroporation Solution. Ultimately, optimal electroporation conditions are dependent on the goal of the

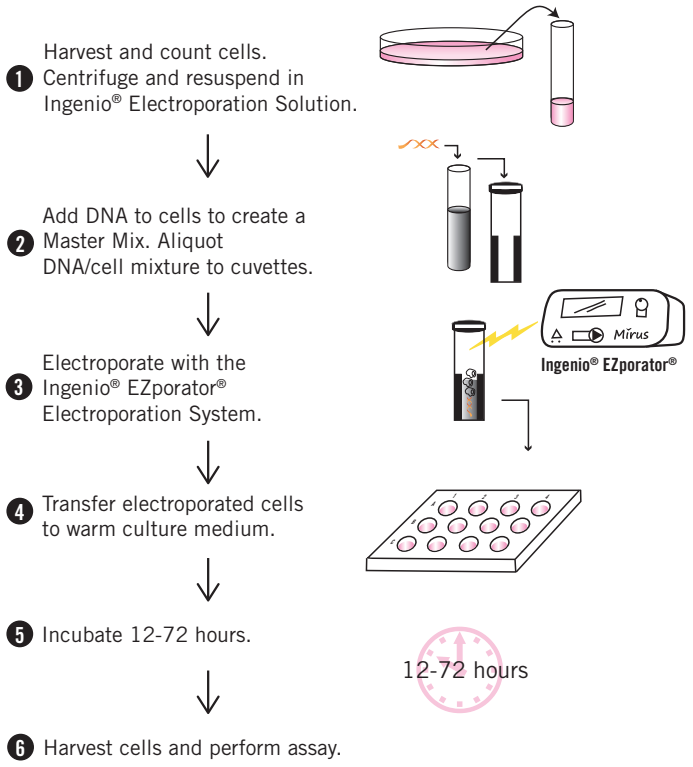
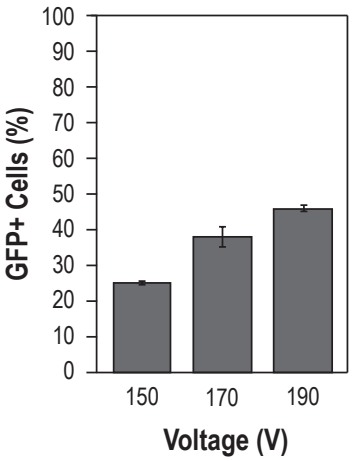


FIGURE 3. General protocol for electroporating cells with the Ingenio® EZporator® Electroporation System.



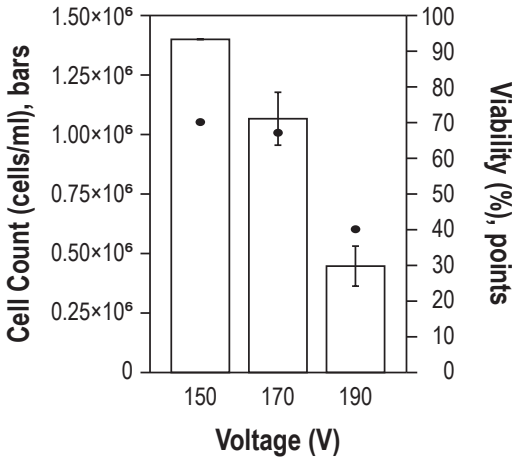


FIGURE 4. Effect of three different voltages on electroporation efficiency, cell count and viability of primary human T cells. Freshly harvested T cells (three days post-activation) were electroporated with an eGFP reporter plasmid and assessed three days post-electroporation by flow cytometry. Electroporation efficiency is shown as the percent of live cells that are GFP+. N=2; mean ± range is shown.

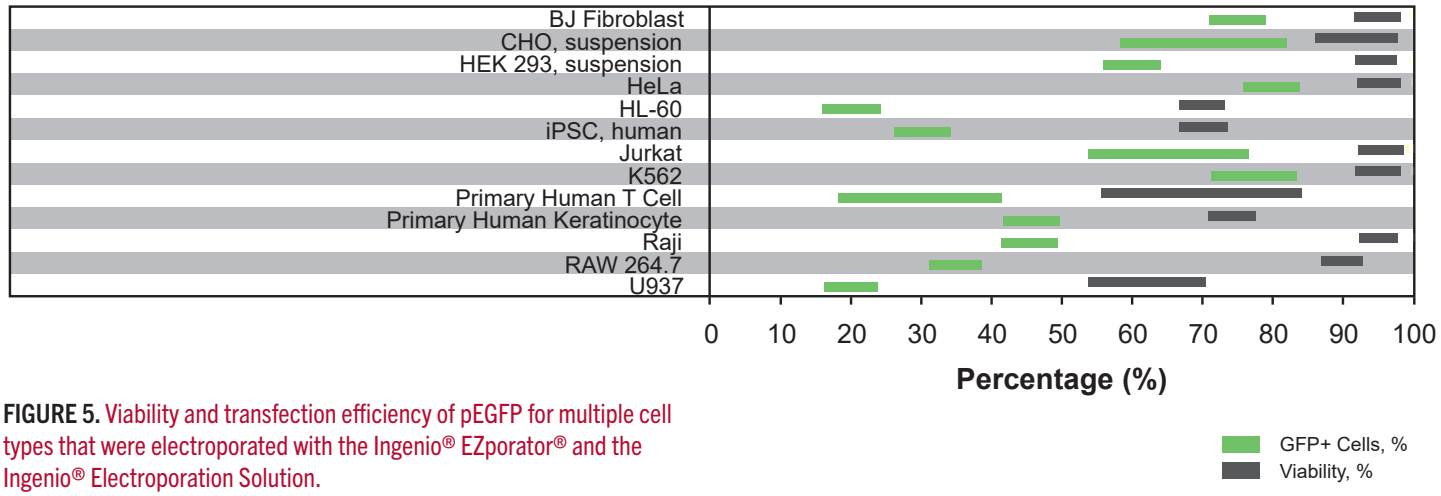


FIGURE 5. Viability and transfection efficiency of pEGFP for multiple cell types that were electroporated with the Ingenio® EZporator® and the Ingenio® Electroporation Solution.

experiment, but the Ingenio® EZporator® platform allows for researchers to adjust them via fine-tuning voltage settings.

We found efficiency of pEGFP electroporation of primary human T cells varied between 15-45% (50-90% viability) using the Ingenio® EZporator®. We concluded that the source of variation is likely dependent on the unique characteristics of primary human T cells such as the donor PBMCs from which they were harvested as well as days since activation. These findings match observations made in recent publications describing optimized electroporation of primary human T cells.[3,4] Additionally, the results with the Ingenio® EZporator® are similar to published as well as manufacturer-listed efficiency and viability obtained by nucleofection™ of plasmid DNA to primary human T cells.[5] However, unlike nucleofection™, which utilizes fixed programs with masked pulse settings, electroporation parameters on the Ingenio® EZporator® are not concealed from the user and can be optimized by simply adjusting voltage.

ELECTROPORATE MULTIPLE CELL TYPES WITH HIGH EFFICIENCY AND VIABLITY

In addition to primary human T cells, we evaluated electroporation efficiency with the Ingenio® EZporator® System for other difficult-to-transfect cell types as well as commonly used cell lines. The electroporation efficiency of an eGFP-reporter plasmid and percentage of viable cells were assessed by flow cytometry 24-72 hours post-electroporation for each cell type. **Figure 5** shows the range of electroporation efficiency (as the percent of live cells that are GFP+) and viability obtained from separate electroporations using the Ingenio® EZporator® and Ingenio® Electroporation Solution.

DELIVER DIFFERENT MOLECULAR CARGO

The efficiency of delivery that can be achieved through chemical transfection and viral transduction is often limited by the size and type of the molecular cargo. While electroporation is also subject to these constraints, the efficiency of delivery is less discriminate to the identity of the cargo that passes through the nanometer-sized pores that are formed during and after electrical pulses are applied.[2] In addition to plasmid DNA, the Ingenio® EZporator® System can deliver other biomolecules, including siRNA and Cas9 ribonuclear protein complexes (Cas9 RNPs), making gene knockdown, stable transgene expression, and gene-editing possible for cell types difficult to modify via other methods.

As shown in **Figure 6**, Cas9 RNP complexes targeting PPIB (cyclophilin B) were successfully delivered into primary human T cells using the Ingenio® EZporator® System. Cas9 RNP complexes were composed of PPIB-targeting two-part gRNA and Cas9 protein at a 1:1 ratio of gRNA to Cas9 protein (i.e. 750 nM gRNA and 750 nM Cas9 protein). The primary human T cells were suspended in a 100 µl volume (10×10^6 cells/ml) of Ingenio® Electroporation Solution with the RNP complex in 0.2 cm cuvettes and electroporated at 150 V with the Ingenio® EZporator®. As a negative control, cells without RNP complex were also electroporated at 150 V. At 72 hours post-electroporation, over half of the cells (52.9%, n=2) were edited at the intended locus as measured by a T7E1 mismatch detection assay and capillary electrophoresis. Here we show that primary human T cells, which are difficult to genetically modify using non-viral methods, can be edited efficiently through electroporation with the Ingenio® EZporator® System.

CONCLUSIONS

The Ingenio® EZporator® paired with the Ingenio® Electroporation Kits and Solution comprises a simple system for non-viral gene delivery to diverse cell types, including difficult-to-transfect cells such as primary T cells. Furthermore, relatively high cellular viability is achievable with proper optimization of applied voltage, which is a setting that can be easily adjusted on the Ingenio® EZporator® at 2 V increments in the 20-400 V range recommended for mammalian and insect cell types. This easy-to-use electroporation system is a cost-effective, complementary approach to using chemical transfection reagents and viral vectors for basic and applied research.

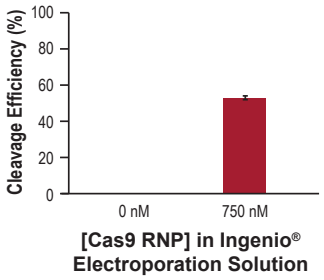


FIGURE 6. Freshly harvested human T cells were electroporated with Cas9 RNP complexes. A T7E1 mismatch detection assay was used to measure cleavage efficiency at 72 hours post-electroporation. N=2; mean ± range is shown.

REFERENCES

(1) Weaver JC and Chizmadzhev YA. Theory of electroporation: A review. *Bioelectrochemistry and Bioenergetics*. 1996; 41(2): 135-160.

(2) Kotnik T, *et al.* Membrane Electroporation and Electropermeabilization: Mechanisms and Models. *Annual Review of Biophysics*. 2019; 48(1): 63-91.

(3) Zhang Z, *et al.* Optimized DNA Electroporation for Primary Human T Cell Engineering. *BMC Biotechnology*. 2018; 18(1): 4.

(4) Aksoy P, *et al.* Viable and efficient electroporation-based genetic manipulation of unstimulated human T cells. *bioRxiv.* 2019; 466243; doi: https://doi.org/10.1101/466243.

(5) Chicaybam L, *et al.* An Efficient Low Cost Method for Gene Transfer to T Lymphocytes. *PLoS ONE*. 2013; 8(3): e60298.

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