Ingenio® Electroporation Solution - The High Efficiency, Broad Spectrum Solution For Nucleic Acid Delivery into Primary Cells and Hard to Transfect Cell-lines

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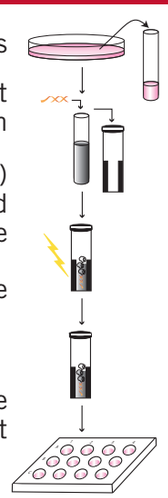
Abstract

Primary cells as well as certain cell-lines are known to be refractory to traditional chemical transfection methods. The physical technique of electroporation has emerged as a method of choice for nucleic acid delivery into these "hard to transfect" cells. Ingenio® Electroporation Solution is a broad spectrum reagent capable of supporting electroporation of plasmid DNA and siRNA into multiple mammalian cell lines. Ingenio is compatible with most standard electroporators including Lonza-amaxa® Nucleofector®, Bio-Rad Gene Pulser Xcell™ and Harvard-BTX® electroporators. Cells can be efficiently transfected using a simple protocol with Ingenio Electroporation Solution, that can be further optimized for either exponential decay or square wave pulse types offered on standard electroporators. Certain cell types transfect better with square wave pulses while others respond better to exponential decay; empirical testing is required for each cell type. Regardless of the type of pulse used, optimization of pulse strength is absolutely critical in ensuring high efficiency electroporation without loss of cell viability. Best electroporation results are achieved using Ingenio Electroporation Solution by titrating the pulse variables such as voltage and capacitance. Electrotransfection using optimized pulse parameters with Ingenio Electroporation Solution or Kits affords increased gene expression or knockdown in primary cells and several different "hard to transfect" cell lines, with minimal cytotoxicity.

General Methods



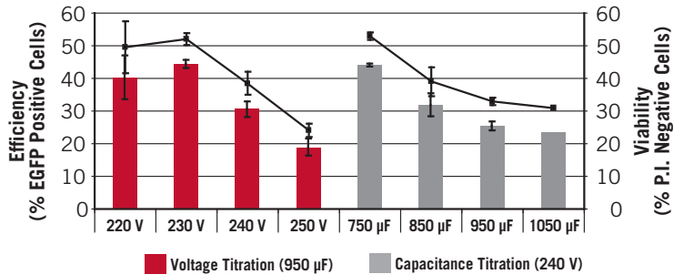
Optimize Ingenio® Electroporation

- **Pulse Type:** Exponential Decay, Square Wave
- **Pulse Conditions:** Voltage, Capacitance, Resistance, Time, Pulse Interval
- **Nucleic Acid Concentration**
- **Cell Density**

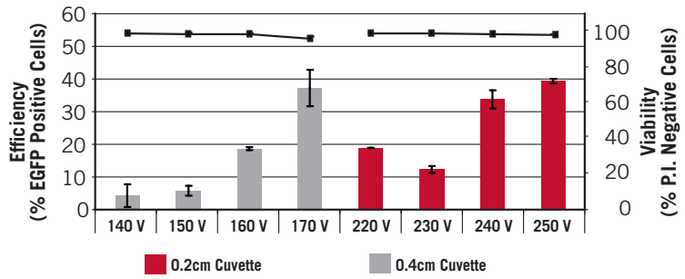
Results and Discussion

Ingenio Protocol Optimization

1A. Pulse Condition Optimization - Exponential Decay

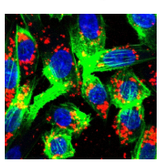


1B. Pulse Condition Optimization - Square Wave

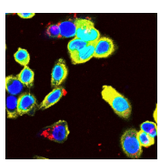


Pulse Condition Optimization: Electroporations performed using (1A) exponential decay pulse in SK-N-MC cells in 0.4 cm cuvettes on Gene Pulser™ Xcell Eukaryotic System (Bio-Rad) and (1B) square wave pulse in primary Mouse Embryonic Fibroblasts in 0.2 cm and 0.4 cm cuvettes using ECM 830 (BTX™). Exponential decay pulse titration was performed by varying voltage keeping capacitance constant and vice-versa. For square-wave optimization, voltage was varied at a fixed capacitance of 950µF. EGFP efficiency and viability assay of Propidium Iodide stained cells were performed using BD™ LSRII flow cytometer at 24 hours post-electroporation.

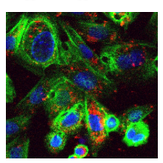
Delivery of Plasmid DNA and siRNA Using Ingenio™



2A. Plasmid Delivery: Cy™3-labeled control plasmid DNA



2B. Plasmid Expression: Cy™3-labeled plasmid expressing EYFP protein (yellow)

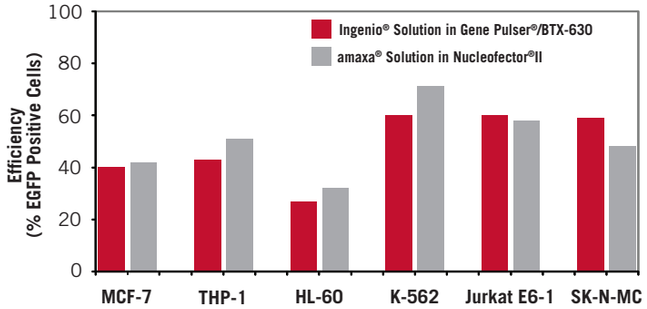


2C. siRNA Delivery: Cy™3-labeled noncoding control siRNA

Twenty-four hours post-electroporation with Ingenio, CHO-K1 cells were fixed and counterstained with TO-PRO®-3 and Alexa Fluor 488® phalloidin (Invitrogen). Confocal images were recorded using Zeiss LSM510 confocal microscope using lasers set at 488nm, 550nm and 633nm wavelengths. Images were analyzed by Z-stack AxioVision software (Zeiss) to determine intracellular localization.

High Efficiency Plasmid Delivery into Hard to Transfect Cells

3A. Achieve Efficiencies Similar to amaxa® Using Ingenio®



3B. Efficient Delivery of Plasmid DNA into Primary Cells

Primary Cells	Ingenio® (in ECM 630, GenePulser)						Ingenio® (in Lonza-amaxa® NucleofectorII)		
	Exp. Decay Pulse (V) (950µF)		Efficiency		Viability		amaxa Program#	Efficiency	Viability
	0.2 cm	0.4 cm	0.2 cm	0.4 cm	0.2 cm	0.4 cm			
Mouse Embryonic Fibroblast	150	230	35%	40%	65%	70%	T-020	40%	70%
Human Keratinocyte	150	220	21%	23%	62%	67%	T-018	29%	66%

Transfecting "Hard to Transfect" Cell-lines and Primary Cells: (3A) Electroporation using Ingenio in BioRad Gene Pulser Xcell™ or BTX ECM 630 electroporator yields comparable efficiencies to amaxa® Nucleofector® Solution V in Lonza-amaxa NucleofectorII. (3B) Primary cells successfully electroporated in ECM 630, GenePulser and amaxa NucleofectorII using Ingenio Solution while maintaining high viability. Cells were assayed at 24 hours by flow cytometry and reported as percentage of live cell population.

Conclusions

Ingenio® Electroporation Solution and Kits

- High efficiency delivery of plasmid DNA or siRNA into primary cells and hard to transfect cell-lines
- Multi-platform compatible with any electroporation instrument including Bio-Rad Gene Pulser Xcell™, amaxa® Nucleofector®II and BTX® ECM 630 & 830
- Simple and straight-forward protocol with optimization steps for delivering nucleic acids with exponential decay or square wave pulse type
- Cost effective and reliable method of delivery into primary cells and cell-lines