

The Gene Pulser MXcell™ Electroporation System Provides Reproducible Results in Electroporation Plates and Cuvettes With the Same Protocol

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Introduction

The Gene Pulser MXcell is a versatile electroporation system that performs transfections either in a multiwell plate or in a cuvette. The Gene Pulser MXcell plate chamber accepts multiwell electroporation plates, allowing testing of up to 24 different sets of conditions in a single experiment to achieve rapid and simple optimization of the electroporation conditions. In addition, the small well volume of the 96-well electroporation plate (~150 μ l) is convenient when working with a small number of cells. This is particularly advantageous when working with samples such as primary cells where cell numbers are often limiting. The Gene Pulser MXcell™ ShockPod™ chamber accepts standard electroporation cuvettes, and is ideal when performing a small number of electroporations or when a single cuvette format is desired.

Typically, switching among cuvette sizes requires re-optimizing electroporation conditions to retain the same transfection efficiency because several parameters affecting the electrical field change simultaneously. The field strength of the electrical pulse is equal to the voltage divided by the cuvette gap distance in centimeters. Therefore, the field strength can be adjusted when switching between a 0.4 cm and a 0.2 cm cuvette by halving the voltage; however, the transfection efficiencies will likely differ between the cuvettes because other parameters, such as sample resistance, also vary. Resistance varies with cell density, path length, and contact area between the electrode and buffer, making exact replication of conditions difficult when moving between cuvette sizes.

In this tech note we demonstrate that with the Gene Pulser MXcell system, it is possible to use 0.4 cm electroporation cuvettes or 96-well plates interchangeably both for square and exponential waveforms. The same electroporation settings achieve comparable results with either a 0.4 cm electroporation cuvette or a 96-well plate as long as the volume used in the 0.4 cm cuvette is equal to the total volume of one well set in the 96-well plate (one well set is a column of four wells).

The ability to interchange the reaction vessels without re-optimizing settings makes it possible to rapidly and easily optimize conditions using the 96-well electroporation plate, and then utilize those optimal conditions in experiments with electroporation cuvettes or plates.

Methods

Cell Cultures

Chinese hamster ovary (CHO) cells were cultured in Ham's F12K medium supplemented with 10% fetal bovine serum (FBS). COS-7 cells were cultured in GIBCO DMEM (Invitrogen Corporation) supplemented with 10% FBS. Cells were passaged the day prior to electroporation to ensure they were actively growing on the day of the experiment.

Electroporation

Prior to electroporation, the cells were harvested by trypsinization, washed with PBS, counted, and resuspended in Gene Pulser® electroporation buffer (Bio-Rad Laboratories, Inc.) at a density of 1×10^6 cells/ml. Plasmid DNA with the green fluorescent protein (GFP) reporter gene (gWIZ GFP mammalian expression vector, Genlantis, Inc.) was added to the cell suspensions at a final concentration of 20 μ g/ml. The cells were then transferred to either 96-well electroporation plates or 0.4 cm electroporation cuvettes and pulsed with the Gene Pulser MXcell electroporation system (Bio-Rad). Four replicate well sets and four cuvette electroporations were performed for each condition. Control samples were taken from the cell suspensions, but were not electroporated.

Electroporation conditions varied between CHO and COS-7 cells but were kept identical between the cuvette and the electroporation plate for each cell type. CHO cells were electroporated using a square-wave pulse of 250 V and 2000 μ F capacitance for 20 ms with a parallel resistance of 1000 Ω . The COS-7 cells were electroporated with an exponential-decay pulse of 210 V and 450 μ F capacitance with a parallel resistance of 1000 Ω . For both cell types, each of the four wells of a well set in the 96-well electroporation plate contained 150 μ l of cell suspension or electroporation buffer. Accordingly, 600 μ l (4 x 150 μ l) of cell suspension was used for the cuvettes.

After electroporation, the cells were resuspended and aliquots were transferred into prewarmed growth medium and incubated at 37°C for 24 hr. After 24 hr, transfection efficiency and cell viability were determined by flow cytometry. The culture dishes were washed with PBS to remove dead cells and cellular debris, then cells were trypsinized and transferred to a round bottom tube. Cells were pelleted by centrifugation, then resuspended to known volume in PBS with the addition of CountBright absolute counting beads (Invitrogen). Transfection efficiency was determined by the number of cells expressing GFP relative to the total number of cells. Cell viability was assessed by comparing the number of cells in the experimental treatments to cell number in control samples after normalizing cell number to number of beads.

Results and Discussion

In order to compare the electroporation success achieved when using the MXcell plate chamber and the MXcell ShockPod cuvette chamber, both the CHO and COS-7 cell suspensions were electroporated using 96-well electroporation plates and 0.4 cm electroporation cuvettes. These two cell lines were chosen because we have previously demonstrated that the best transfection efficiencies for CHO cells (Terefe et al. 2008) and COS-7 cells are obtained using square and exponential waveforms, respectively. Electroporation success was determined by flow cytometry 24 hr after electroporation. Electroporation achieved transfection efficiencies of approximately 70% for the CHO cells and 72% for the COS-7 cells (Figure 1). For both cell lines, comparable numbers of cells expressing GFP were found between samples electroporated in cuvettes and in 96-well electroporation plates (Figure 1). Differences in transfection efficiency between cuvettes and plates averaged only 1.3% for CHO cells and 0.4% for COS-7 cells; both differences were within the standard deviations among replicates. Therefore, the choice of delivery vessel did not affect the transfection efficiency using either the square-wave pulse (CHO cells) or the exponential-decay pulse (COS-7 cells).

To achieve similar transfection efficiencies between the two cell lines required using conditions that were more detrimental to the viability of the COS-7 cells. Under the electroporation conditions used for the respective cell types, the COS-7 cells averaged 45% viability with a standard deviation of 8% and the CHO cells averaged 72% viability with a standard deviation of 11%.

The electric field in the electroporation vessel and the resulting transfection efficiency and cell survival can vary

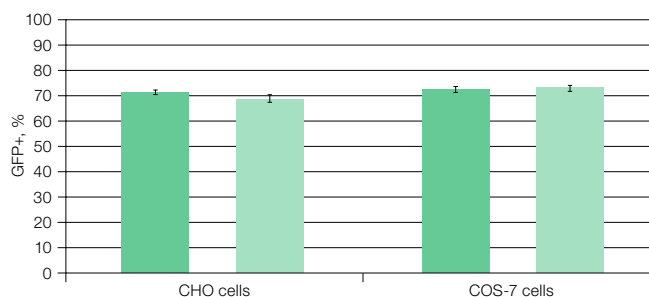


Fig. 1. Transfection efficiencies of CHO and COS-7 cells after electroporation. Cells were electroporated in 96-well electroporation plates (■) or 0.4 cm electroporation cuvettes (□) using either a square-wave protocol (CHO) or exponential-decay protocol (COS-7). Expression of GFP reporter gene was monitored by flow cytometry 24 hr after electroporation. Each bar represents the mean of four replicate electroporations. Error bars indicate standard deviations.

with a number of parameters. Despite these challenges, the Gene Pulser MXcell system provides consistent results not only among replicates but also when switching between electroporation cuvettes and plates. Our results show that by simply adjusting the volume used in the 0.4 cm electroporation cuvette to equal the volume of one well set of the 96-well electroporation plate, it is possible to achieve the same transfection efficiency using identical electroporation parameters.

Conclusions

The ability to switch between multiwell electroporation plates and standard electroporation cuvettes adds flexibility to the versatile Gene Pulser MXcell electroporation system. Since the same conditions determined for multiwell plates can be applied, cuvettes provide an economical alternative once optimal conditions have been established. Switching between electroporation vessels requires only that the total volume used in each well set equals the volume in a cuvette. For example, 150 µl per well requires 600 µl per cuvette and 200 µl per well converts to 800 µl per cuvette. In addition, our results show that comparable transfection efficiencies can be obtained for cuvettes and multiwell plates regardless of the waveform used.

Reference

Terefe J et al. (2008). Electroporation conditions for Chinese hamster ovary cells using the Gene Pulser MXcell™ electroporation system. Bio-Rad Bulletin 5704.

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