# Electroporation of Primary Murine Mast Cells Using the Gene Pulser MXcell™ Electroporation System

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## Introduction

The use of primary cells — cells isolated directly from tissues or blood — is rapidly becoming the desired model system for examining physiological processes. Transfection of these cells provides researchers with a powerful means of examining a multitude of cellular processes in vitro, as well as allowing ex vivo studies such as cell tracking analyses. In contrast to immortalized cell lines, these cells typically undergo minimal cell division and have a finite lifespan in culture, making gene transfer and expression a challenge.

In order to study primary cells, suitable tissue culture conditions to generate and/or maintain the cell of interest must first be developed. Typically, mature primary cells, or immature precursors, are harvested from an animal and then grown ex vivo as a cell culture. Hematopoietic cells are particularly well suited to growth and differentiation by culturing them in vitro and many different types of blood cells can be generated from bone marrow. One such hematopoietic cell, the mast cell, is particularly amenable to ex vivo differentiation and is comparatively hardy and long-lived (Itakura et al. 2001, Razin et al. 1984). Mast cells play a fundamental role in asthma and allergy, and as such, these cells represent an excellent primary cell model.

Electroporation is a fast and adaptable method to introduce exogenous nucleic acid into primary cells. Here, we report the use of the Bio-Rad Gene Pulser MXcell electroporation system to successfully transfect mast cells. Results shown here demonstrate that using this approach, mast cells can be transfected with high efficiency and low cytotoxicity.

## Methods

### Cell Culture

Primary mast cell cultures were established from the bone marrow of 8–12 week BALB/c mice (The Jackson Laboratory). Bone marrow was flushed from the femurs and tibiae and cells were cultured at 1 x $10^6$ cells/ml in GIBCO RPMI Media 1640 (Invitrogen Corporation) supplemented with 10% fetal calf serum, 1% L-glutamine, 1% penicillin/streptomycin, and β-mercaptoethanol. Interleukin-3 (IL-3) (10 ng/ml, BD Biosciences) was added at the initial plating, and every seven days thereafter to promote differentiation to mast cells. Stem cell factor (20 ng/ml, PeproTech, Inc.) was added after 14 days, and every 7 days thereafter. The medium was changed weekly. After 5 weeks in culture, mature mast cells were fully differentiated and ready for transfection.

### Electroporation

Mast cells were washed once with PBS, counted, and suspended at 1 x $10^7$ cells/ml in Gene Pulser® electroporation buffer (Bio-Rad Laboratories, Inc.). Plasmid DNA with the green fluorescent protein (GFP) reporter gene (gWIZ GFP mammalian expression vector, Genlantis, Inc.) was added to the cells at a final concentration of 20 µg/ml. Subsequently, the cell suspension was transferred into the wells of a 96-well electroporation plate (Bio-Rad) and pulsed using the Gene Pulser MXcell electroporation system (Bio-Rad). To determine the optimal electroporation conditions for mast cells, both exponential-decay and square-wave pulses were tested using a variety of settings.

After electroporation, the cells were transferred into tissue culture plates containing prewarmed GIBCO RPMI Media 1640 (Invitrogen) with 10 ng/ml IL-3 and incubated for 24 hr at 37°C. Transfection efficiency and cell viability were determined by flow cytometry analysis 24 hr postelectroporation. The cells were stained with propidium iodide (PI) prior to flow cytometry analysis. Transfection efficiency was expressed as the number of cells expressing GFP relative to the total number of live cells. Cell viability was assessed using PI staining.

## Results and Discussion

Square-wave pulses were found to be more effective than exponential-decay pulses at delivering plasmid DNA to mast cells while maintaining cell viability (Figure 1). The highest transfection efficiencies were obtained with a square-wave protocol delivering a 15 ms pulse at 350 V; this reproducibly yielded ~30% transfection rates with ~50% cell viability. As illustrated in Figure 1, higher cell viabilities can be obtained; however, this is at the cost of reduced transfection efficiency.

We tested the effect of cell density on electroporation efficiency. Cell densities of 1 x $10^6$, 5 x $10^6$, and 1 x $10^7$ cells/ml yielded similar transfection efficiencies and cell viabilities (Figure 2).


**Fig. 1. Transfection efficiency and viability of mast cells after electroporation.** Cells were electroporated using exponential-decay and square-wave pulses with different parameters. Expression of reporter GFP (top charts) and cell survival (bottom charts) were monitored 24 hr postelectroporation. The best parameters are indicated by an asterisk. Values are the mean of three replicate experiments.

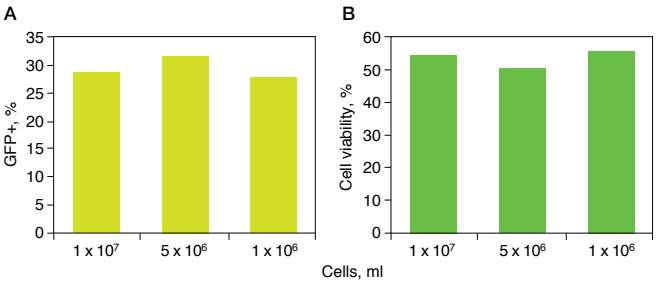


**Fig. 2. Transfection efficiency (A) and cell viability (B) of electroporated mast cells at three cell densities.** All experiments were carried out at 350 V, with a pulse duration of 15 ms. Values are the mean of three replicate experiments.

Expression of GFP was examined at 24 hr and 48 hr postelectroporation. At both time points, comparable levels of GFP expression relative to living cells were observed, thus illustrating that these electroporation conditions are suitable for extended time point cell analyses (Figure 3).

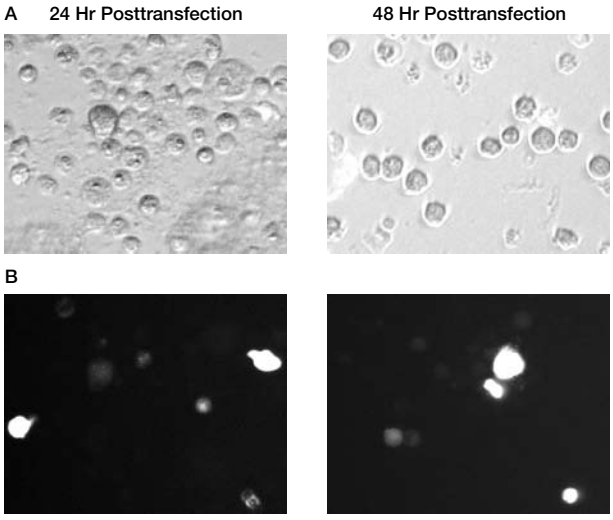


**Fig. 3. Microscopic observation of mast cells after transfection.** **A,** contrast phase microscopy images of cells; **B**, corresponding fluorescence microscopy images of cells expressing GFP.

## Conclusions

Using the Gene Pulser MXcell electroporation system we have been able to rapidly optimize electroporation conditions for primary mast cells. Our results indicate that after a 15 ms pulse at 350 V using a square-wave protocol, roughly one-third of the cells express the reporter gene (GFP). Depending upon experimental needs, electroporation conditions can be modified to increase either cell viability or transfection efficiency.

This work demonstrates the utility of the Gene Pulser MXcell electroporation system in the optimization of electroporation conditions for primary cells. Rapid optimization is particularly valuable when working with primary cells, as conditions may vary substantially for each cell type due to differences in size, granularity, and replicative state. Furthermore, primary cells are often not available in large numbers and are not long-lived. Therefore, simultaneous examination of numerous electroporation conditions using small numbers of cells greatly reduces the amount of cell culture required to obtain sufficient cell numbers for analysis.

## References

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