# Gene Pulser MXcell™ Electroporation System

## Instruction Manual

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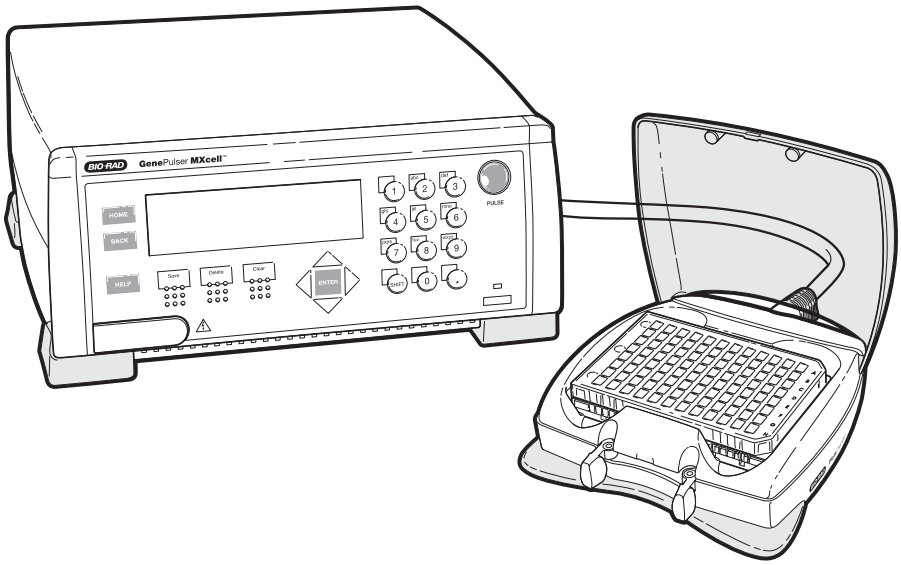


BIO-RAD

# Gene Pulser MXcell™ Electroporation System

## Instruction Manual

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# Safety and Regulatory Compliance

The Gene Pulser MXcell™ electroporation system is designed to run safely. Please read the following sections to learn about the safe use of this system and regulatory requirements.

General Safety Information

This Bio-Rad instrument is designed and certified to meet the safety requirements of EN61010 and the EMC requirements of EN61326 (for Class A) and conforms to the “Class A” standards for electromagnetic emissions intended for laboratory equipment applications. This instrument is intended for laboratory application only. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or in the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

No part of the Gene Pulser MXcell system should be used if obvious external case damage has occurred or the electronic displays are not functioning as described in the manual. This instrument is only to be used with the components provided (or their authorized additions or replacements) including, but not limited to, supplied cables and plate chamber. The operating temperature range for the Gene Pulser MXcell system and its associated components is 18–35°C.

There are no user serviceable parts within the unit. The operator should make no attempt to open any case cover or defeat any safety interlock. This instrument must not be altered or modified in any way. Alteration of this instrument will result in the following:

- Void the manufacturer’s warranty
- Void the IEC 1010 safety certification
- Create a potential safety hazard

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended or by modification of the instrument not performed by Bio-Rad or an authorized agent.

## Electrical Hazards 

The Gene Pulser MXcell system produces voltages up to 500 V and is capable of passing very high currents. When charged to maximum voltage, the instrument stores about 210 J. A certain degree of respect is required for energy levels of this order. System safety features prevent operator access to the high voltage and to the recessed electrode contacts inside the sample chamber. These mechanical interlocks should never be circumvented.

The PULSE button is active whenever the character space in the lower right corner appears. There is high voltage present whenever the PULSE button is depressed and “pulse being delivered” is shown on the LCD display on the front of the instrument. Because of the built-in safety interlock in the Gene Pulser MXcell plate chamber, no pulse is delivered to the electroporation plate when the plate chamber lid is opened. If the capacitor has been partially charged but not fired (for example, when the charging cycle has been interrupted before the pulse is delivered), some charge may remain on the internal capacitor. This charge will dissipate over 1–2 minutes. However, the user cannot make contact with any charged electrical components due to the system safety features.

## Mechanical Hazards

The Gene Pulser MXcell system contains a patented arc-protection circuit that dramatically reduces the incidence of arcing in the cuvette when high voltage is delivered into the sample. The unit incorporates a circuit that senses the beginning of an arc and diverts current from the sample within <2 µs, preventing or greatly reducing mechanical, visual, and auditory phenomena at the plate chamber. Should an arc occur, the sample chamber is effective in containing these small discharges. If you prefer you can wear safety glasses as additional protection when using the instrument.

## Other Safety Precautions

Avoid spilling any liquids onto the apparatus. Use only a paper towel or a cloth wetted with either water or alcohol to clean the outside surfaces of the Gene Pulser MXcell electroporation system.

Use only the Bio-Rad cables supplied with the Gene Pulser MXcell electroporation system

Use the Gene Pulser MXcell plate chamber only in the assembled condition. Do not attempt to circumvent the protection of the plate chamber or use it while disassembled.

Read the instruction manual before using the Gene Pulser MXcell electroporation system. For technical assistance contact your local Bio-Rad office, or in the US call 1-800-4BIORAD (1-800-424-6723).

> **WARNING!** The Gene Pulser MXcell electroporation system generates, uses, and radiates radio frequency energy. If it is not used in accordance with the instructions given in this manual, it may cause interference with radio communications. The Gene Pulser MXcell has been tested and found to comply with the limits for Class A computing devices (pursuant to Subpart J of Part 15 of FCC Rules) which provide reasonable protection against such interference when operated in a commercial environment. Operation of this equipment in a residential area is likely to cause interference. In this case the user will be required, at his/her expense, to take whatever measure may be required to correct the interference.

# Operating Conditions

To safely operate the Gene Pulser MXcell electroporation system, keep environmental conditions within the following limits:

- Operate between 18°C and 35°C at 90% maximum humidity
- Store between -40°C and +65°C
- Mains voltage is 90-132 VRMS and 198-264 VRMS at 47-63 Hz; auto-select
- Maximum Input Power is 600 VA

# Warranty

The Gene Pulser MXcell electroporation system is warranted against defects in materials and workmanship for 1 year from date of purchase. If any defects occur in the instruments or accessories during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts at its discretion without charge. This warranty does not apply to the fuses and the following items are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Damage caused by substituting alternative parts.
4. Use of fittings or spare parts supplied by anyone other than Bio-Rad Laboratories.
5. Damage caused by accident or misuse.
6. Damage caused by disaster.
7. Corrosion caused by improper solvent or sample.

For any inquiry or request for repair service, contact Bio-Rad Laboratories and tell us the model and serial number of your instrument.

IMPORTANT: This Bio-Rad instrument is designed and certified to meet EN61010* and the EMC requirements of EN61326 (for Class A) safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will cause the following results:

- Void the manufacturer's warranty
- Void the EN61010 safety certification
- Create a potential safety hazard

Bio-Rad Laboratories is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent.

*EN61010 is an internationally accepted electrical safety standard for laboratory instruments.

# Table of Contents

# 1 Introduction

Congratulations on the purchase of a Gene Pulser MXcell™ electroporation system! This instrument is the newest addition to Bio-Rad Laboratories' powerful electroporation line. This system is filled with features that will enable you to quickly optimize conditions for efficient delivery of molecules into most eukaryotic cells, including mammalian cells and plant protoplasts.

The Gene Pulser MXcell electroporation system is designed to electroporate cells in 96-, 24- and 12-well electroporation plates.

## Overview of the Gene Pulser MXcell™ Electroporation System

The system includes three components:

- **Power module:** The power module produces controlled exponential or square waveform pulses. The unit is capable of producing pulses of up to 500 V in a self-contained unit requiring no peripheral modules. This ensures delivery of highly reproducible electroporation conditions to multiwell plates. The system is designed to allow you to vary any of the following parameters: waveform, resistance, voltage, capacitance, pulse duration, and number of pulses.
- **Plate chamber:** The plate chamber holds a variety of multi-well electroporation plates to provide maximum flexibility.
- **Multi-well electroporation plates:** The electroporation plates come in three formats: 12, 24, and 96-well. When using the 96-well plate or 24-well plates, 24 different

conditions can be programmed for use each time; the 12-well plate, 12 conditions. You can use the 96-well plate to optimize conditions, and then perform laboratory scale experiments in the 24- or 12-well plates. Pre-set optimization protocols assist you in selecting initial starting conditions, even when cell lines are new to your laboratory.

# Bio-Rad Resources and References

Bio-Rad Laboratories provides many resources for scientists. The following web sites contain useful information about running electroporation experiments:

- **Gene Expression Gateway (www.bio-rad.com/genomics/)**

  This site provides rich technical resources on a wide variety of methods and applications related to electroporation and gene expression. This site also features tools, citations, technical support, and troubleshooting resources.

- **Life Science Research web site (discover.bio-rad.com)**

  This site includes links to technical notes, manuals, product information, and technical support.

Click the following links to download or request a copy of this manual or other Bio-Rad Laboratories literature:

- Click the PDF icon to download a portable document format copy and open it using Adobe Acrobat Reader software (www.adobe.com).
- Click the folder icon and order a printed copy.
- Click the FAX icon to request a FAX copy.
- Phone your local Bio-Rad Laboratories office to request a printed copy. In the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the Literature option.

Use the following resources to locate what you need:

**Table 1. Bio-Rad resources.**

| Resource | How to contact |
|---|---|
| Local Bio-Rad Laboratories representatives | Find local information and contacts on the Bio-Rad Laboratories web site by selecting your country on the home page (www.bio-rad.com). Also find the nearest international office listed on the back of this manual. |
| Technical notes and literature | Go to the Gene Expression Gateway (www.bio-rad.com/genomics/) and locate the Search box in the upper, right corner of the web page. Type a search term in the box to find links to products, technical notes, and manuals. |
| Technical specialists | Bio-Rad Laboratories provides quality technical support. We staff our Technical Support department with experienced scientists to provide our customers with practical and expert solutions. To find technical support on the web, go to the Gene Expression Gateway (www.bio-rad.com/genomics).<br><br>To find local technical support, contact your nearest Bio-Rad Laboratories office. For technical support in the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the technical support option. |

# Writing Conventions Used in This Manual

This manual is for scientists and technicians who run the Gene Pulser MXcell electroporation system and accessories. It explains how to safely set up and operate the Gene Pulser MXcell system. This manual also contains important tips about how to successfully run electroporation experiments on the Gene Pulser MXcell electroporation system.

This manual uses the writing conventions shown in Table 2 to quickly provide relevant information.

**Table 2. Conventions used in this manual.**

| Convention | Meaning |
|---|---|
| **TIP:** | Provides helpful information and instructions |
| NOTE: | Provides important information, including information explained in further detail elsewhere in this manual |
| **WARNING!** | Explains very important information about something that might damage the researcher, an instrument, or cause data loss |
| **Screen** message | Indicates an LCD screen message or a command that you select or type. For example, "select **Protocol Set-up** in the home screen" means highlight the word "**Protocol Set-up** located in the list in the home screen |
| **NAME** of control panel key | A word in capital letters and Courier font indicates the name of a key on the Gene Pulser MXcell electroporation system control panel. For example, these keys have the following names:<br>• The **ENTER** key is the key named ENTER on the control panel<br>• The **RIGHT** arrow key is the arrow key that points to the right |
| Select **X** | Select **X** by pressing the arrow keys. The word "select" means to press the arrow keys to highlight the word. For example, "select **Protocol Set-up**" means "use the arrow keys to highlight the **Protocol Set-up** option on the LCD screen" |
| Select **X > Y** | From menu **X**, select **Y**. For example, "Select **Protocol Set-up** > **WHOLE PLATE**" means highlight the **Protocol Set-up** option and then select **WHOLE PLATE** in the next screen. In general, the word "select" means to highlight the word on the screen. For example, "Select **WHOLE PLATE**" means press the arrow keys to highlight the word **WHOLE PLATE** on the screen |
| Press **X** | Press **X** key on the control panel. For example, "press **ENTER**" means "Press the **ENTER** key on the control panel" |

For information about safety labels used in this manual and on the Gene Pulser MXcell electroporation system, see "Safety and Regulatory Compliance" on page iii.

# 2 Get Started

The Gene Pulser MXcell™ electroporation system ships as complete system that is ready to setup and start. This chapter provides two sections with information about getting started with this electroporation system:

- Unpacking the Gene Pulser MXcell system (page 5)
- Setting up the system (page 6)
- Introduction to the Gene Pulser MXcell electroporation system (page 8)
- Well sets and quadrants in Gene Pulser electroporation plates (page 12)

For more information about programming and running electroporation experiments on the Gene Pulser MXcell system, see “Program and Run the System” on page 19.

## Unpacking and Setting Up the System

Your Gene Pulser MXcell system shipment includes these components in the package:

- Gene Pulser MXcell power module
- Plate chamber
- Gene Pulser electroporation plate (1 x 96-well)
- Gene Pulser MXcell electroporation system manual
- Protocol quick guide
- Optimization quick guide

Remove all packing material and place components on a flat, dry surface near an appropriate electrical outlet. Please check that all items were shipped. If any items are missing or damaged, contact your local Bio-Rad Laboratories office (page 2).

# Setting Up the Gene Pulser MXcell System

To set up the Gene Pulser MXcell power module, follow these instructions:

1. Attach the power cord to the back of the Gene Pulser MXcell power module (Figure 1):

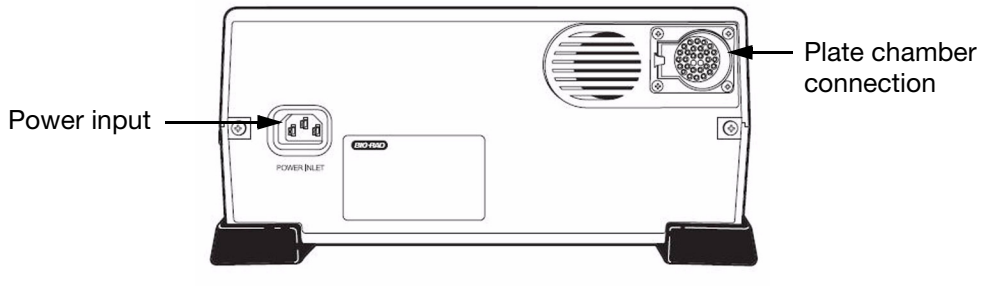


**Figure 1. Rear panel of Gene Pulser MXcell power module.**

2. Connect the plate chamber by plugging the black connector into the back of the power module:

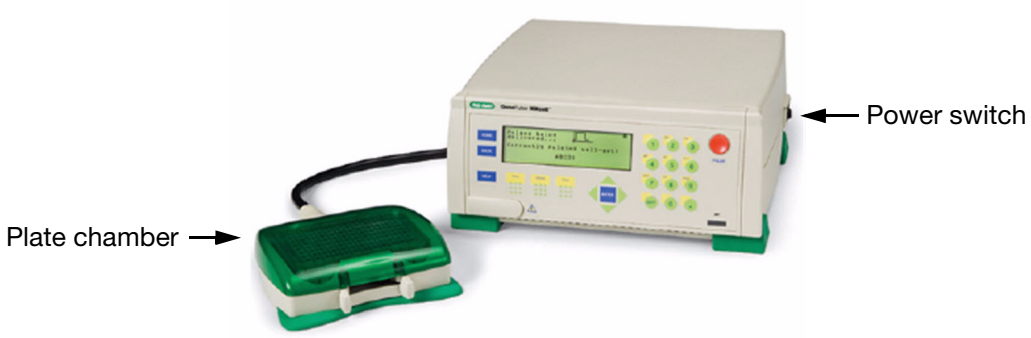


**Figure 2. Front of power module with plate chamber and power switch.**

3. Plug the unit into an appropriate electrical outlet.
4. Turn on the Gene Pulser MXcell system, by pressing the power switch on the right side of the power module (Figure 2).

   **TIP:** Change the angle of the LCD screen by pulling down the foot under the front of the Gene Pulser MXcell system.

5. Begin any operation by selecting an option in the list on the home screen (Figure 3)

   1. Protocol Set-up
   2. Gradient Protocol
   3. User Protocols
   4. Pre-set Protocols
   5. Last Pulse
   6. Data Management
   7. Screen Intensity
   8. Measurements

**Figure 3. Home screen options.**

For more information about the options listed in the home screen, see page 19.

The plate chamber holds the Gene Pulser electroporation plates. The top lid of the chamber must be closed to use the plate chamber to deliver a pulse. The safety design of the system requires that the top be closed before a pulse is applied. No pulse is delivered to the electroporation plate when the chamber lid is open.

NOTE: The electroporation plate slot will only accept the Gene Pulser MXcell electroporation plates designed specifically for this instrument.

Follow these steps to operate the plate chamber:

1. Squeeze the tabs on the front of the plate chamber to release the latch and to open the top.


**Figure 4. Opening the plate chamber.**

2. Insert a 96-, 24- or 12-well plate, line up the pins, and push down firmly.

**Figure 5. Plate chamber with inserted 96-well electroporation plate.**

3. To close the chamber, gently push the top down.

   NOTE: Use only low-resistance media such as GPEB (<1000 ohms) with the Gene Pulser MXcell system.

# Introduction to the System

Your Gene Pulser MXcell electroporation system is designed for ease of use and intuitive programming. This section provides an overview of how to operate the system:

- Overview of Gene Pulser MXcell system (page 8)
- Using the control panel and keys (page 9)
- Selecting operations in the home screen (page 10)

## Overview of the Gene Pulser MXcell Electroporation System

The Gene Pulser MXcell power module provides one of two distinct waveforms in a pulse:

- Exponential waveform (EXP)
- Square waveform (SQR)

To deliver a waveform, the power module contains a set of capacitors with a functional range between 25 and 2475 µF that is selected in 25 µF increments. For square wave pulses, the power module provides the large capacitance (2,475 µF) necessary for delivering the pulse into low resistance media.

The power module selects an electronically controlled resistance of 50 to 1500 Ω. The module controls the resistance of the circuit by placing resistors in parallel with the sample, thereby providing a means of reducing the time constant of an exponential decay pulse. This method provides an effective means of controlling the time constant when using higher-resistance media, but has little effect on the time constant when using low-resistance media.

> NOTE: Only use low-resistance media such as GPEB (<1000 W) with the Gene Pulser MXcell system.

The Gene Pulser MXcell electroporation system uses Gene Pulser electroporation plates in 12-, 24-, and 96-well formats that are specially designed for use with this system (see “Well Sets and Quadrants in Electroporation Plates” on page 12).

Following a pulse, the results for each plate or well set display on the screen.

> **TIP:** Visit the Gene Expression Gateway tools web page (www.bio-rad.com/genomics/) to download a template to records your results.

When the Gene Pulser MXcell system delivers a pulse, it can use the following parameters:

- Voltage in volts (V)
- Current in microfarads (µF)
- Resistance in ohms (Ω)
- Duration in milliseconds (ms)
- Number of pulse is the number of individual pulses (NP)
- Pulse interval is the time between each pulse

## Using the Control Panel

The control panel includes an LCD screen and a keypad:

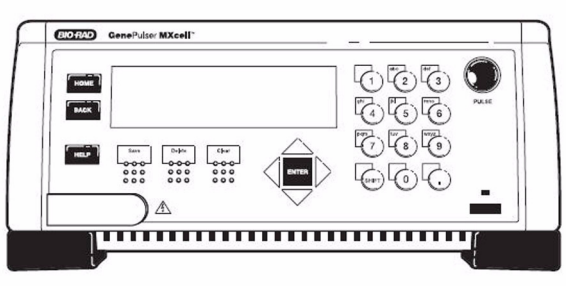


**Figure 6. Gene Pulser MXcell system control panel with keypad.**

Use the keys the Gene Pulser MXcell control panel to enter all parameters for an electroporation experiment. Table 3 describes the function of each key on the control panel:

**Table 3. Function of the keys on the control panel.**

| Keys | Function |
|---|---|
| Alphanumeric | Press these keys to enter letters or numbers when programming the Gene Pulser MXcell system. Press the **Shift** key to toggle between alphabetic and numeric entries. To enter the same key twice, advance the cursor using the right arrow key. For example to type “a” and then “b”, press the “abc” key, press **ENTER**, and then press the “abc” key twice.<br>NOTE: The second digit in a two-digit number must be entered within 2 seconds after the first digit. If the second digit is entered too late the power module will enter a single-digit number rather than a two-digit number. |
| **Shift** | Toggle between alphabetic and numeric entries. For example, to type an alphabetic character, press the **Shift** key to enter alpha mode, then press the key with the appropriate letter. To type an “a”, press the 2 key once, and to type a “b”, press the 2 key twice, to type a c, press the 2 key three times. In general, the Gene Pulser MXcell system automatically changes between alphabetic and numeric input if only one type of input is needed. |
| **HOME** | Return to the home screen from anywhere in the program |
| **BACK** | Return one level back in toward the home screen within any operation. |
| **HELP** | Displays context-sensitive help for each operation screen. To open the help screen, press the **HELP** key. To leave the help screen and return to the current operation, press the **HELP** key again.<br><br>Each help screen describes the keys you press to enter the next function, and continue the current operation. Press the up and down arrow keys to scroll through the help screens. |
| **Save** | Saves user names and user protocols. |
| **Delete** | Removes only the last entry in the field; also used to remove User Name and User Protocol files |

**Table 3. Function of the keys on the control panel.**

| Keys | Function |
|---|---|
| **Clear** | Remove the entire line of the field. |
| **ENTER** | Press this key to confirm a selection or to move the cursor to the next location. |
| Arrow | Press one of the four arrow keys to move the cursor in the direction of the arrow. Up and down Arrow keys move the cursor up or down one row at a time. Depending on the screen and location of the cursor, the right and left Arrows will move the cursor to the right or left one space at a time, toggle forward and backward one screen when there are multiple screens for the same menu, or increase or decrease numerical input values. |
| **PULSE** | Pressing this red key initiates an electric pulse. When a pulse is delivered to the plate, the screen displays **Pulsing** and the system emits a sound. When the system delivers multiple pulses, a sound emits after the last pulse is delivered. |

## Overview of Options in the Home Screen

The Gene Pulser MXcell system home screen includes all the options you need:

- **Program and run protocols:** Define the parameters of your electroporation experiment.
- **System functions:** View data, test your buffer, or adjust the screen.

The home screen provides access to all the operations in the system, including options to program protocols:

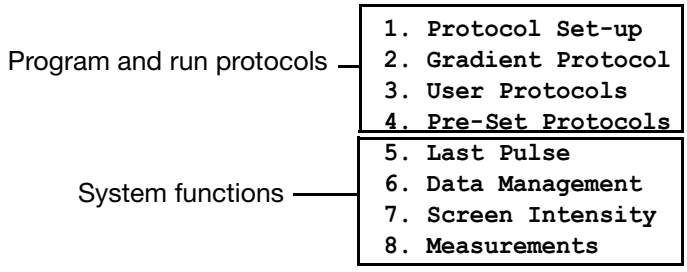


**Figure 7. The home screen options.**

**TIP:** To return to the home screen from anywhere in the program, press the **HOME** key on the control panel.

The options include two types of operations:

- **Protocols:** Use one of the four protocol options to create, store, or open protocols to run with any of the Gene Pulser electroporation plates. Within a plate, well sets and quadrants can be programmed to run separate protocols. Within each protocol the parameters can vary for each individual well, each well set, or each quadrant.
- **System functions:** Use one of four functions to access data or adjust system parameters.

To begin an operation on the Gene Pulser MXcell system, select one of the options in the home screen. Table 4 lists all the options and the associated operations.

**Table 4. Options listed in the home screen.**

| Option | Operation |
|---|---|
| **PROTOCOLS** | |
| **Protocol Set-up** | Manually program the parameters that will be delivered to the plate. |
| **Gradient Protocol** | Specify initial values that will be used to automatically generate a gradient of settings across all the wells on the plate. |
| **User Protocols** | Access all protocols within the directory of each system user and create new user directories. |
| **Pre-Set Protocols** | Open one of eighteen pre-set protocols designed for easy optimization of parameters to deliver to the plate during a pulse. These protocols can be modified and saved with a different name. |
| **SYSTEM FUNCTIONS** | |
| **Last Pulse** | Recall the electroporation parameters for the last pulse and to deliver a pulse using the same conditions. |
| **Data Management** | View pulse parameters and results for the last 100 pulses logged by date and time. |
| **Screen Intensity** | Adjust the contrast intensity of the LCD display. |
| **Measurements** | Measure the resistance or capacitance of any well on this device. |

# Well Sets and Quadrants in Electroporation Plates

The Gene Pulser electroporation plates are available in three different formats: 96-well, 24-well and 12-well. Table 5 shows the recommended cell concentration and volume for each well in a plate:

**Table 5. Electroporator plate formats.**

| Plate format | Cell concentration | Volume | Number of well sets |
|---|---|---|---|
| 96-well | 1 x $10^5$ to 2 x $10^6$ | 100-200 ul | 24 |
| 24-well | 5 x $10^5$ to 8 x $10^6$ | 500-800 ul | 24 |
| 12-well | 1 x $10^6$ to 1.5 x $10^7$ | 1.0-1.5 ml | 12 |

For more information about how to electroporate mammalian cells, see “Preparation of Mammalian Cells” on page 15.

Well sets and quadrants divide the electroporation plates into functional units. You have the option of running a different protocol in each different well set or quadrant. Each plate format is divided according to these definitions:

- **Well set:** A group of wells within a plate
- **Quadrant:** All the wells or well sets in one quarter of a plate

## Well sets

A well set is a group of four wells in a column of a 96-well plate in which programmed electroporation conditions are delivered simultaneously. Well sets can assist you in performing the following:

- Replicating experiments by using the same type of cells and same protocol
- Testing different variables under identical electroporation conditions by putting different experiments (molecules or cells) in different well sets. For example, deliver different siRNAs into the same cell line.

In a 96-well plate, each well set is composed of 4 adjacent wells in a column. For example column 1 with rows A, B, C, and D is one well set (Figure 8):

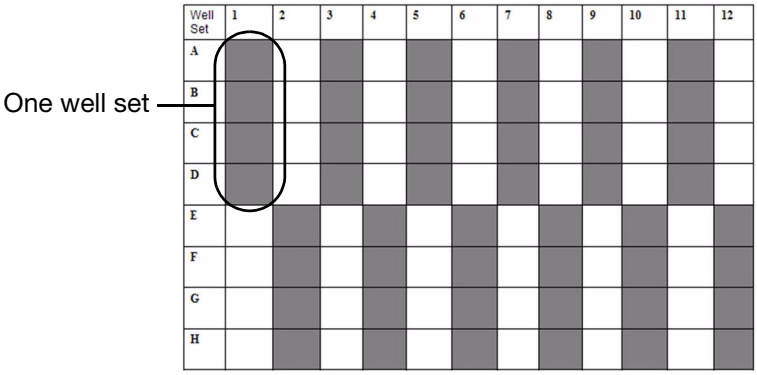


**Figure 8. Well sets in a 96-well plate.**

In Figure 8, the shaded and unshaded areas represent sets of wells that are grouped together in a well set. During a run, the parameters entered for each well set are simultaneously delivered to all the wells in that set.

When programming a protocol, the well set appears with the letters of the rows, followed by the column number. For example the well set “ABCD1” includes wells A, B, C, and D in column 1 (Figure 8).

**WARNING!** All wells in a well set must be filled with either sample or sample buffer. For example, if you want to electroporate six wells, fill a complete well set (such as ABCD1) with sample and fill two wells in a second well set (such as AB2) with sample. Finally, be sure to fill the remaining two wells in the second well set (such as CD2) with the sample buffer.

## Quadrants

The 24- and 96-well plates can be programmed in quadrants. A quadrant in a 96-well electroporation plate includes a group of six well sets. In a 24-well plate each quadrant consists of six groups of wells. Programming quadrants is an easy way to replicate gradient experiments. Figure 9 shows the quadrants in both the 96-well and 24-well electroporation plates.

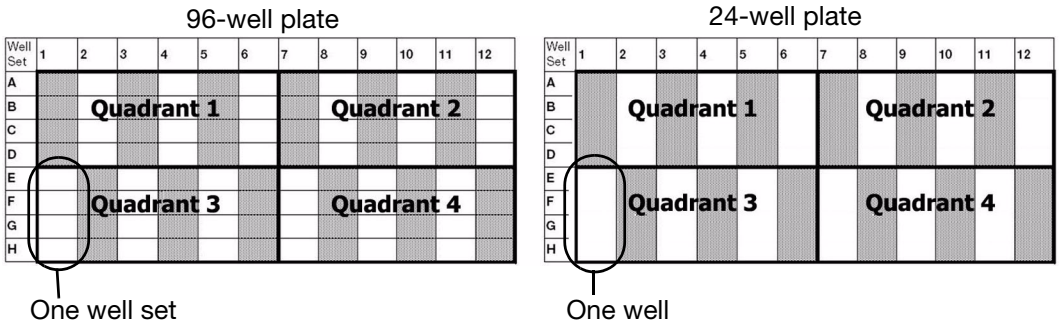


**Figure 9. Quadrants in 96- and 24-well plates.**

# 3 Prepare Mammalian Cells

Preparation of cells is critical to the success of any electroporation experiment. This chapter contains important information about how to run a successful experiment on the Gene Pulser MXcell™ electroporation system. Refer to the following sections:

- Electroporation of Mammalian cells (page 15)
- Reagents and Solutions for Electroporation (page 17)

## Preparation of Mammalian Cells

This section describes techniques for preparing electrocompetent mammalian cells, and how to manipulate them after electroporation. Many of these techniques have been tested at Bio-Rad Laboratories. The following list provides an overview of the major steps to run an electroporation experiment using the Gene Pulser MXcell™ electroporation system system. Refer to the pages listed to obtain more information.

- Harvest and count the cells (“Harvesting and Counting the Cells” on page 16).
- Prepare the cells for electroporation (“Preparing the Cells for Electroporation” on page 16).
- Resuspend cells in Gene Pulser electroporation buffer and transfer them to the electroporation plate (“Electroporation” on page 16)
- Insert electroporation plate into plate chamber (figure 5 on page 7)
- Create a new protocol, or select an existing protocol from the home screen and press the **PULSE** button to electroporate the cells (“Protocol Set-Up Option” on page 20)
- Remove the electroporation plate and plate the cells in fresh cell culture plates. If needed, combine the cells from different wells into the same culture plate. Assess the transfection efficiency. (“Assessing Transfection Efficiency” on page 17)

For information about the factors that affect electroporation conditions, see page 41.

## Harvesting and Counting the Cells

To harvest and count cells, follow these instructions:

1. Passaged the cells the day before electroporation. All cell types should be harvested when they are actively growing. If working with adherent cells, trypsinize the cells to detach them. Add growth media and then pellet the cells. If working with suspension cells, pellet the cells.
2. After pelleting the cells, remove the media and wash the cells once with PBS by gently pipeting them.
3. Remove an aliquot from the washed cells and count the cells.

## Preparing the Cells for Electroporation

To prepare the cells for electroporation, follow these instructions:

1. Aliquot the number of cells needed to perform the experiment. For adherent cells, we recommend using 1 x $10^6$ cells/ml, but we have successfully used 0.5-5 x $10^6$ cells/ml. For suspension cells we recommend 2-3 x $10^6$ cells/ml, but we have successfully used 1-5 x $10^6$ cells/ml.
2. Pellet the cells. Aspirate the PBS and resuspend the cells in the appropriate volume of Gene Pulser electroporation buffer reagent (1 ml per 1 x $10^6$ of adherent cells, and 1 ml per 2-3 x $10^6$ of suspension cells).
3. Add the nucleic acid or other molecule. For siRNA electroporation, use 10-100 nM of siRNA. For plasmid DNA electroporation, use 5-40 µg/ml.

## Electroporation

To electroporate cells, follow these instructions:

1. Use 100-200 µl of mixture (cells in electroporation buffer reagent with nucleic acid) per well of a 96-well electroporation plate. Use 500-800 µl of mixture per well of a 24-well electroporation plate. Use 1-1.5 ml of mixture per well of a 12-well electroporation plate.

   **WARNING!** All wells in a well set must be filled with either sample or sample buffer. For example, if you want to electroporate six wells, fill a complete well set (such as ABCD1) with sample and fill two wells in a second well set (such as AB2) with sample. Finally, be sure to fill the remaining two wells in the second well set (such as CD2) with the sample buffer.
2. Rock the plate to wet the electrode and distribute the cells evenly
3. Transfer cells to tissue culture dishes containing growth media.
4. Incubate cells at 37 °C in a humidified $CO_2$ incubator until ready to be assayed.
5. After incubating 24 hours change the growth media.

## Assessing Transfection Efficiency

To assess the efficiency of a transfection experiment, several techniques can be used: Fluorescently labeled siRNAs can be used to determine the transfection efficiency for siRNA delivery. Transfection efficiency can be measured by fluorescence microscopy or by flow cytometry. For plasmid delivery the transfection efficiency can be determined by electroporating plasmids expressing reporter genes such as GFP or β-galactosidase.

If you electroporate a mammalian cell line for the first time, choose growth conditions following the electroporation that are based on other experiments with those cells or with other similar cells. Reagents and Solutions for Electroporation

This list includes recommended reagents and solutions for running an electroporation experiment:

- Gene Pulser electroporation buffer (catalog #165-2676 and 165-2677)
- Growth medium with FBS and necessary additives
- Trypsin-EDTA
- Sterile PBS: 137 mM NaCl, 2.7 mM KCl, 9.5 mM sodium phosphate, pH 7.3

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# 4 Program and Run the System

The Gene Pulser MXcell™ electroporation system runs experiments with Pre-Set protocols, or with protocols that you create. To run an electroporation experiment, you can either program a new protocol or select an existing protocol.

This chapter includes instructions for programming protocols, running protocols, or using other system functions:

- Turn on the Gene Pulser MXcell system (page 20)
- Select the **`Protocol Set-up`** option to program new protocols in the whole plate or in well sets (page 20)
- Select the **`Gradient Protocol`** option to program a new gradient protocol in the whole plate or in quadrants (page 22)
- Select the **`User Protocols`** option to open user protocols (page 24)
- Select **`Pre-Set Protocols`** option to open pre-set protocols (page 25)
- Open the **`Last Pulse`** option to view the last pulse that was delivered (page 26)
- Open the **`Data Management`** option to view data from delivered protocols (page 26)
- Select the **`Screen Intensity`** option to change the LCD brightness (page 27)
- Select the **`Measurements`** option to check buffer resistance and capacitance (page 27)
- Save a new protocol or an edited protocol (page 28)
- Add a new user with the **`User Protocols`** option (page 29)

# Turning on the System

To turn on the Gene Pulser MXcell system, press the power button on the right side of the power module (Figure 2 on page 6).

Once initiated, the Gene Pulser MXcell system runs a series of tests. These test verify that the system is running within specifications. The tests check the PulseTrac™ system (page 49) and the firmware. During the tests the system displays the Bio-Rad Laboratories logo, the Gene Pulser MXcell system name, and the firmware version.

After initialization, the home screen displays (page 10). From this screen it is easy to access all options, including the first four options for programming and running protocols. To select an option in the home screen, press the up and down arrow keys to select an option, and press **ENTER** to confirm the selection.

> **TIP:** To return to the home screen from anywhere in the program, press the **HOME** key on the control panel.

# Protocol Set-Up Option

The Protocol Set-up option allows you to program protocols for the plate as follows:

- **Whole plate (WHOLE PLATE):** Apply the same pulse parameters to the entire electroporation plate (page 20).
- **Well set (WELL SET):** Apply parameters to one well set within the electroporation plate (page 21).

### WHOLE PLATE PROGRAMMING

To program a whole plate, follow these instructions:

1. **Select `Protocol Set-up` in the home screen.**

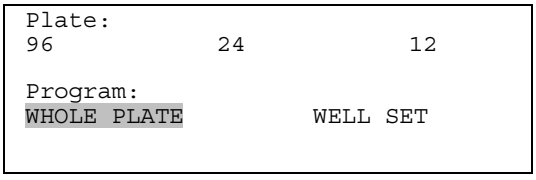
   Then press **ENTER** to confirm the selection.

2. **Select a plate size.**

   Use the arrow keys to select the **Plate** (**96**, **24** or **12**), and press **ENTER** to confirm the selection.

3. **Select WHOLE PLATE.**

   Press **ENTER** to confirm the selection:




4. **Select a waveform.**

   Press the arrow keys to select the waveform (**EXP** or **SQR**), and press **ENTER** to confirm the selection.

5. **Enter the parameters.**

   Enter the required parameter values for the pulse units.

   NOTE: If the values you enter for a parameter is outside the limits of the Gene Pulser MXcell system, the value will change to the closest permitted value.

Press the arrow keys to select a parameter, then press the alphanumeric keys to enter a new value. Press **ENTER** to confirm the entry.

```
96-Well              Whole Plate
Edit well Set?  (press ENTER)
Waveform:  EXP - SQR
       V:              ---
       C:              ----
       R:               ?
```

**TIP:** Press **Clear** to delete the value.

This example shows some values for each parameter in the pulse with a square waveform (**SQR**):

```
96-Well              Whole Plate
Edit well Set?  (press ENTER)
Waveform:  EXP - SQR
       V:              500
       C:              500
       R:               ?
       D:              5.500
       #.  3    S: 10.0          P
```

NOTE: The **P** in the lower right corner indicates that the required parameters are complete, and the **PULSE** button is ready.

**TIP:** You can also edit a well set by selecting **Edit well Set?** using the arrow keys to move the cursor. Press ENTER to begin editing. For more information about well set programming, see Step 5 "Select a waveform (EXP or SQR)." on page 22.

6. **(Optional) Press Save to save the changes in the protocol (page 28).**

7. **Press the PULSE button to electroporate the sample.**

   **TIP:** Once you start a protocol, you can stop the experiment if needed. To stop a protocol, press and hold the **PULSE** button. When the protocol stops, the screen displays the last pulse (see “Last Pulse Option” on page 26).

## WELL SET PROGRAMMING

To program a plate with well sets that run different protocols, follow these instructions:

1. **Select Protocol Set-up in the home screen.**

   Press **ENTER** to confirm the selection.

2. **Select a plate size.**

   Press the arrow keys to select a plate size, then press **ENTER** to accept the selection.

3. **Select WELL SET.**

   Select **WELL SET**, then press ENTER to accept the selection.

```
Plate:
96           24           12

Program:
WHOLE PLATE         WELL SET
```

4. **Select a well set in the plate.**

   Use the left and right arrow keys to select the well set, or to move through parameter. Press **ENTER** to confirm the parameter entry.

5. **Select a waveform (EXP or SQR).**

   Press the arrow keys to select a waveform. Then enter the values for each parameter. Once entered, press **ENTER** to confirm the selection.

   NOTE: The **P** in the lower right corner indicates that the required parameters are complete, and the **PULSE** button is ready.

6. **(Optional) Select another well set.**

   Press, and hold the up and down arrow keys to select another well set in the plate. Enter all the parameters for each well set in the run.

7. **(Optional) Press the PULSE button to electroporate the sample.**

   **TIP:** Once you start a protocol, you can stop the experiment if needed. To stop a protocol, press and hold the **PULSE** button. When the protocol stops, the screen displays the last pulse (see “Last Pulse Option” on page 26).

# Gradient Protocol Option

The Gradient Protocol allows you to specify values that will be used to automatically generate a gradient of settings across all the wells of a plate. This protocol is a quick way to optimize conditions for your specific cell type.

NOTE: Gradient protocols run only on 24- and 96-well electroporation plates.

To set up a gradient protocol, first select the part of the plate to apply the gradient. Follow the instructions in the following sections to set up a gradient protocol in a plate:

- **GRADIENT:** Run a gradient protocol through a well set or the whole plate (page 23)
- **QUADRANT:** Run a gradient protocol through a quarter of the wells in a plate (page 23).

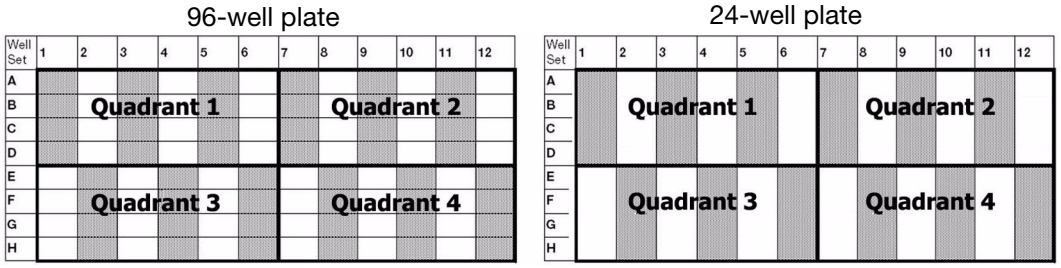



**Figure 10. Quadrants in 96-well and 24-well plates. A quadrant is one quarter of the wells in the plate.**

The median voltage entered is applied to the mid point of the well set. In a whole plate, this corresponds to well set EFGH1, in a quadrant, it is either ABCD4, ABCD10, EFGH4, or EFGH10. Wells to the left of the percent value entered decrease by the percentage entered, while wells to the right of the percentvalue entered increase by the percent. Upon completion of the run, values for each parameter can be obtained by using the right arrow key.

## GRADIENT PROGRAMMING

To program a gradient protocol, follow these instructions:

1. **Select `Gradient Protocol` in the home screen.**

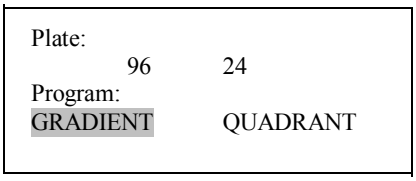
   Press **ENTER** to confirm the selection.

2. **Select a plate size.**

   Press the arrow keys to select the **Plate** (**96** or **24**) and press **ENTER** to confirm the selection.

3. **Select GRADIENT.**

   Select **GRADIENT**, and then press **ENTER** to confirm the selection:

4. **Select a waveform (EXP or SQR).**

   Press the arrow keys to select a waveform, then enter the values for each parameter. Once entered, press **ENTER** to confirm the selection.

   NOTE: The **P** in the lower right corner indicates that the required parameters are complete, and the **PULSE** button is ready

5. **Press the PULSE button to electroporate the sample.**

   **TIP:** Once you start a protocol, you can stop the experiment if needed. To stop a protocol, press and hold the **PULSE** button. When the protocol stops, the screen displays the last pulse (see “Last Pulse Option” on page 26).

## QUADRANT PROGRAMMING

To program a quadrant program, follow these instructions:

1. **Select `Gradient Protocol` in the home screen.**

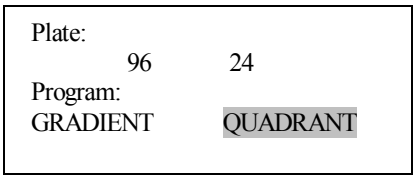
   Press **ENTER** to confirm the selection.

2. **Select a plate size.**

   Press the arrow keys to select the **Plate** (**96** or **24**) and press **ENTER** to confirm the selection.

3. **Select QUADRANT.**

   Press the arrow keys to select **QUADRANT** and press **ENTER** to confirm the selection.

4. **Select a waveform (EXP or SQR).**

   Press the arrow keys to enter the values for each parameter. Once entered, press **ENTER** to confirm the selection.

   NOTE: The **P** in the lower right corner indicates that the required parameters are complete, and the **PULSE** button is ready.

5. **(Optional) Select another quadrant.**

   Press and release the up and down arrow keys to move through parameters within a quadrant. Press and hold the up and down arrow keys to select another quadrant.

6. **Press the `PULSE` button to electroporate the sample.**

   **TIP:** Once you start a protocol, you can stop the experiment if needed. To stop a protocol, press and hold the **`PULSE`** button. When the protocol stops, the screen displays the last pulse ("Last Pulse Option" on page 26).

# User Protocols Option

Choose an existing protocol to run, or create, open, rename, or delete a protocol from the **`User Protocol`** list. Save any protocol as a user protocol for future experiments. Run the protocol, or edit it and then run it.

To select and run a user protocol, follow these instructions:

1. **Select `User Protocols` in the home screen.**

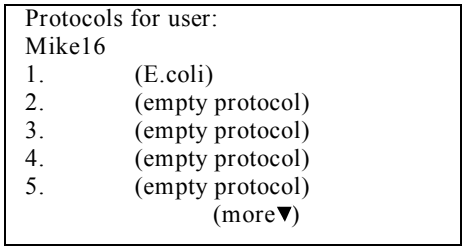
   Press **`ENTER`** to confirm the selection:

   NOTE: This opens the directory for the current user. To select a different user directory, select **User Directory** instead. Press ENTER to confirm selection.

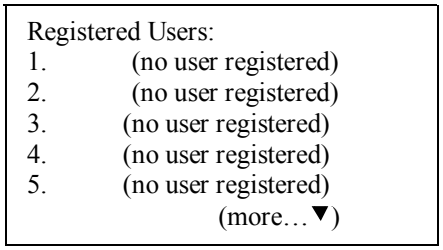
2. **Select for a protocol from the list in the user protocol.**

   Press the up and down arrow keys to select a protocol. Then Press ENTER to confirm selection.

3. **(Optional) To view another protocol from another user's directory, press the `Back` key.**

   Press and release the up and down arrow keys to move from one protocol to the next. Press and hold the up and down arrow keys to scroll from screen to screen.

4. **Edit a selected protocol.**

   Enter the changes and press the **Save** key to save the changes:
   - To rename a protocol, press the CLEAR key to delete it, type in the new name. press ENTER to confirm change.
   - Change parameters of the protocol by selecting the desired parameters and typing new parameters
   - Press ENTER to confirm the selection

5. **Delete a selected protocol.** Press the DELETE key. At the screen prompt, press the DELETE key again.

6. **Press Save to save the changes in the protocol (page 28).**

7. **Press the PULSE button to electroporate the sample.**

   **TIP:** Once you start a protocol, you can stop the experiment if needed. To stop a protocol, press and hold the **PULSE** button. When the protocol stops, the screen displays the last pulse (see “Last Pulse Option” on page 26).

# Pre-Set Protocols Option

Choose an existing protocol to run from the **Pre-Set Protocols** list. Save any protocol as a user protocol for future experiments. Run the protocol, or edit it and then run it.

Bio-Rad Laboratories scientists have developed twenty-one optimized pre-set protocols so you can quickly run an experiment. These protocols optimize typical electroporation conditions, starting with known parameters or starting with unknown parameters.

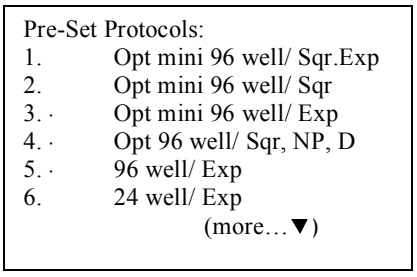
To run a pre-set protocol, follow these instructions:

1. **Select Pre-Set Protocol in the home screen.**

   Press **ENTER** to confirm the selection.

2. **Select for a pre-set protocol from the list.**

   Press and release the up and down keys to move from one protocol to the next. Press and hold the arrow keys scroll from screen to screen. Press **ENTER** to confirm the selection.




   NOTE: For detailed information about the parameters and plate setup for each pre-set protocol, see “Program and Run the System” on page 19.

3. **(Optional) Change the values of the parameters.**

   Press the arrow keys to select the parameter and press the alphanumeric keys to enter a new value. To save the changes and create a new protocol, press the **Save** key (page 28).

   NOTE: You must change the name of the protocol before saving. Press the CLEAR key to delete the Pre-set protocol name, and use the alphanumeric keys to type a new name. (page 28).

4. **(Optional) Press Save to save the changes in the protocol (page 28).**

5. **Press the PULSE button to electroporate the sample.**

   **TIP:** Once you start a protocol, you can stop the experiment if needed. To stop a protocol, press and hold the **PULSE** button. When the protocol stops, the screen displays the last pulse (see “Last Pulse Option” on page 26).

# Last Pulse Option

Once a pulse is completed, the screen displays the last pulse data. You can alsto view the data by selecting the **Last Pulse** function.

**TIP:** This function allows you to proceed from the last pulse the system delivered before a power failure.

To open the **Last Pulse** option, follow these instructions:

1. **Select Last Pulse from the HOME screen**

2. **Press ENTER to confirm the selection.**

# Data Management Option

The data management function stores a list of the last 100 protocols that were run, starting from the most recent and ending with the oldest. Use this list to view the exact parameters of an experiment.

NOTE: When the maximum number of files is reached, the oldest protocol file is deleted.

When you run a protocol, the data management lists the experimental results with the parameters used in that experiment. The name displayed in this list is the name of the protocol used when you ran the experiment.

To view the experiments listed in the data management operation, follow these instructions.

1. **Select Data Management from the HOME screen**

   Press the up and down keys to select **Data Management** option. Then press **ENTER** to accept the selection.

2. **Press the up and down arrow keys to select a protocol in the list.**

   **TIP:** To change the name of the experiment in the data management list, enter a new name when the experiment is selected. Then press **ENTER** to save the new name. At the screen prompt, pres Yes to save or No to return to the previous screen.

3. **Press one of these keys to open or delete the selected experiment:**
   - Press **ENTER** to view the selected protocol.

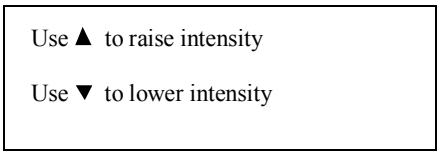
- Press **Delete** to remove the selected protocol from the list.
- At the screen prompt, press Yes to delete or No to return to the previous screen.

# Screen Intensity Option

Adjust the screen intensity when you cannot clearly see the screen. To change the screen intensity, follow these instructions:

1. **Select `Screen Intensity from the HOME screen.`**
2. **Adjust the screen intensity.**

   Press the up and down arrow keys to change the intensity of the screen and view the results of the change:



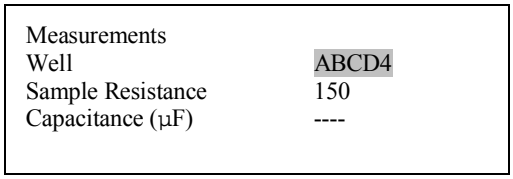

# Measurements Option

Open the Measurements screen to measure the sample resistance of the buffer in your plate. Use this function to verify a buffer, or troubleshoot experimental results.

NOTE: To select the plate size rof measuring buffer resistance, you must access the protocol set-up. Select the plate size, return to the **HOME** screen and follow these instructions:

1. **Select the Measurements option from the HOME screen.**
2. **Enter a well ID to measure the resistance of the sample in that well.**

   Press the left and right arrow keys to select a well to measurement. Then press **ENTER** to accept the selection, press **ENTER** to obtain the measurement.



# Saving Protocols

Once a protocol is programmed or a pre-set protocol is changed, you have the option to save the protocol as new user protocol.

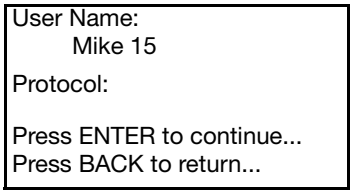
To save a protocol as a file, follow these instructions:

1. **After programming or editing a protocol, press the `Save` key to start saving the protocol.**

2. **Type in a user name by pressing the alphanumeric keys.**

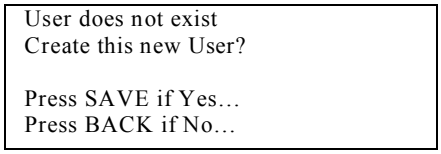
   **TIP:** Press the alphanumeric keys to enter new characters. Press the **`Shift`** key to toggle between letters and numbers. Then press the **`ENTER`** key to save the name. To erase, press the **`Clear`** key.

   In this example the user name is **`Mike 15`**:

3. **(Optional) If the user does not exist, then create a new user.**

   Enter the new user name and press **`ENTER`** to accept the name.

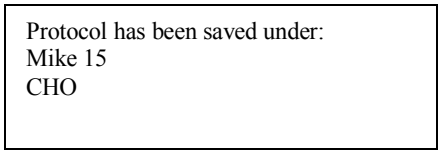
   
   

4. **Enter the name of the Protocol using the alphanumeric keys.**

   **TIP:** Press the alphanumeric keys to enter new characters. Press the **`Shift`** key to toggle between letters and numbers. Then press the **`ENTER`** or **`Save`** key to save the name. To erase press the **`Clear`** key.

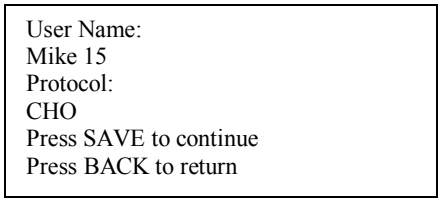
5. **Press the `Save` key to save the protocol.**

   A screen briefly appears verifying the new protocol name:

6. **Press the SAVE key to continue of the BACK key to the protocol.**

   **Tip:** If a protocol is not saved and you proceed to program another protocol or perform other functions in the system, you will be asked if the protocol should be saved or deleted.




# Adding and Deleting the Users and Protocols

The Gene Pulser MXcell system stores user protocol files in a directory with the user name. Add a new user before saving a protocol, or automatically add the user while saving a protocol. Users can also be deleted

NOTE: The user directory holds up to 15 protocols under 20 user names for a total of 300 entries.

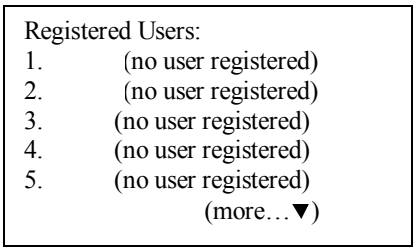
To add a user name, follow these instructions:

1. **Select User Protocols from the home screen.**

   Press **ENTER** to confirm the selection.

2. **Select User Directories.**

   Press the arrow keys and the **ENTER** key to select **User Directories**. The screen displays a list of registered users:




   NOTE: Up to 20 users can be registered; the screen display 5 at a time.

   **TIP:** Press and release the up and down arrow deys to move from one user name to the next. Press and hold the up and down arrow keys to scroll from screen to screen.

3. **Select a number that lists "no user registered."; press ENTER to confirm selection.**

4. **Type the name using the alpha-numeric keypad.**

   To change from letters to numbers press and release the **Shift** key. To confirm the new user name press the **ENTER** key.

5. **Press Save to save the user in the next screen.**

To delete a user name, follow these instructions:

1. **Select User Directory and press the DELETE key.**

2. **At the screen prompt, press Yes to delete or No to return to the previous screen.**

# 5 Pre-Set Protocols

The Gene Pulser MXcell™ electroporation system provides a large set of pre-set protocols developed by scientists at Bio-Rad Laboratories. Use these protocols to quickly run an optimization experiment.

The pre-set protocols have been named using these rules: Each begins with a three to eight letter description and type of electroporation plate (12, 24, or 96-well), followed by a forward slash and the type of waveform (**EXP** or **SQR**). The end of the name lists the specific parameter values with the abbreviated names.

The lists and tables in this chapter use abbreviations for the protocol names and parameters:

- Voltage in volts (V)
- Current in microfarads (µF)
- Resistance in ohms (Ω)
- Duration in milliseconds (ms)
- Number of pulse is the number of individual pulses (NP)
- Pulse interval is the time between each pulse (s)
- **Grad** is a gradient
- **Exp** is exponential waveform
- **Sqr** is square waveform

Refer to the page numbers listed in Table 6 (starting on page 32) for more information about each set of protocols.

**Table 6. List of pre-set protocols and their uses.**

| Protocol name | | Number of wells | When to use this protocol |
|---|---|---|---|
| **Protocols for well sets with four or six wells (page 34)** | | | |
| 1. | Opt mini 96 well/ Sqr.Exp | 24 | Use to rapidly determine optimal waveform and conditions. |
| 2. | Opt mini 96 well/ Sqr | 24 | Use to rapidly to determine optimal conditions for square-wave protocols |
| 3. | Opt mini 96 well/ Exp | 24 | Use to rapidly determine optimal conditions for exponential protocols. |
| 4. | Opt 96 well/ Sqr, NP, D | 16 | Use after optimal square-wave conditions have been determined to enhance specifically cell viability and improve efficiency. |
| **Whole plate protocols (page 36)** | | | |
| 5. | 96 well/ Exp | 96 | Use for initial protocol set-up for many cell types. |
| 6. | 2 4well/ Exp | 24 | Use for initial protocol set-up for many cell types. |
| 7. | 12 well/ Exp | 12 | Use for initial protocol set-up for many cell types. |
| 8. | 96 well/ Sqr | 96 | Use for initial protocol set-up for many cell types. |
| 9. | 24 well/ Sqr | 24 | Use for initial protocol set-up for many cell types. |
| 10. | 12 well/ Sqr | 12 | Use for initial protocol set-up for many cell types. |
| **Well set protocols (page 37)** | | | |
| 11. | 96 well/ Exp,Vgrad/ Cgrad | 96 | Use when working with new cells lines that traditionally apply exponential waveforms. This protocol fine tunes conditions and includes replicates. |
| 12. | 96 well/ Sqr,Vgrad/ Dgrad | 96 | Use when working with new cells lines or when square-waveform protocols are normally applied. This protocol fine tunes conditions and includes replicates. |

**Table 6. List of pre-set protocols and their uses.**

| Protocol name | | Number of wells | When to use this protocol |
|---|---|---|---|
| **Mixed protocols (page 38)** | | | |
| 13. | Opt 96 well/ Exp,Sqr | 96 | Use with cell lines with no protocol reference. This protocol includes a range of average starting conditions. |
| 14. | Opt 24 well/ Exp,Sqr | 24 | Use with cell lines with no protocol reference. This protocol includes a range of average starting conditions. |
| 15. | Opt 12 well/ Exp,Sqr | 12 | Use with cell lines with no protocol reference. This protocol includes a range of average starting conditions. |
| 16. | Uniform 96 well/ Exp,Sqr | 96 | Use with a set of defined conditions to compare different cell lines and electroporation of different molecules within the same or different cell lines. |
| 17. | Uniform 24 well/ Exp,Sqr | 24 | Use with a set of defined conditions to compare different cell lines and electroporation of different molecules within the same or different cell lines. |
| 18. | Uniform 12 well/ Exp,Sqr | 12 | Use with a set of defined conditions to compare different cell lines and electroporation of different molecules within the same or different cell lines. |
| 19. | Mixed 96 | 96 | Use for mixing different waveforms. Alternating rows of exponential (250 V/350 uf) and square waves (250 V/20 ms). |
| 20. | Mixed 24 | 24 | Use for mixing different waveforms. Alternating rows of exponential (250 V/350 uf) and square waves (250 V/20 ms). |
| 21. | Mixed 12 | 12 | Use for mixing different waveforms. Alternating rows of exponential (250 V/350 uf) and square waves (250 V/20 ms). |

# Mini-Optimization Protocols

The following pre-set protocols use four or six well sets in the electroporation plate.

## Opt mini 96 well/ Sqr.Exp

Table 7 shows the parameters that vary in each well set in a 96-well plate. The protocol includes the following well sets:

- **Well set ABCD 1-3:** Square wave, 200 V, 2,000 µF, 1,000 Ω, and 20 ms
- **Well set ABCD 4-6:** Exponential wave, 250 V, 350–750 µF, and 1000 Ω

**Table 7. Opt. mini 96/ Sqr.Exp conditions that vary across the plate.**

| | **Square wave** | | | **Exponential wave** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| **A** | 200 V<br>2,000 µF<br>20 ms | 250 V<br>2,000 µF<br>20 ms | 300 V<br>2,000 µF<br>20 ms | 250 V<br>350 µF<br>1000 Ω | 250 V<br>500 µF<br>1000 Ω | 250 V<br>750 µF<br>1000 Ω | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

## Opt mini 96 well/ Sqr

Table 8 shows the parameters that vary in each well of a 96-well plate. The protocol includes the following well sets:

- **Well set ABCD 1-3:** Square wave, 200–300 V, 2000 µF, 1000 Ω, 20 ms
- **Well set ABCD 4-6:** Square wave, 250V, 2000 µF, 1000 Ω, 15-25 ms

**Table 8. Opt mini 96well/sqr conditions that vary across the plate.**

| | Square wave | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | 200 V<br>20 ms | 250 V<br>20 ms | 300 V<br>20 ms | 250 V<br>15 ms | 250 V<br>20 ms | 250 V<br>25 ms | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

## Opt mini 96 well/ Exp

Table 9 shows the parameters that vary in each well of a 96-well plate. The protocol includes the following well sets:

- **Well set 1-3:** 200–300 V, 350 µF, and 1000 Ω
- **Well set 4-6:** 250V, 200–500 µF, 1000 Ω

**Table 9. Opt mini 96 well/exp conditions that vary across the plate.**

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 200 V<br>350 µF | 250 V<br>350 µF | 300 V<br>350 µF | 250 V<br>200 µF | 250 V<br>350 µF | 250 V<br>500 µF | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

## Opt 96 well/ Sqr, NP, D

Table 10 shows the parameters that vary in each well of a 96-well plate. The protocol includes well sets 1–4 with these parameters: Square Wave, 250 V, 2,000 µF, 1000 Ω, 7–20 ms, and 1–3 pulses (NP).

**Table 10. Opt 96well/ Sqr, NP, D conditions that vary across the plate.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 20 ms<br>1 NP | 15 ms<br>2 NP | 10 ms<br>2 NP | 7 ms<br>3 NP | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

# Whole Plate Protocols

Whole plate protocols program all the wells in an electroporation plate with the same conditions. The following is a list of the protocol names and conditions for each of the whole plate protocols:

- **96 wells/ xp:** Exponential wave, 250 V, 350 µF, and 1,000 Ω
- **24 wells/ Exp:** Exponential wave, 250 V, 350 µF, and 1,000 Ω
- **12 well/ Exp:** Exponential wave, 250 V, 350 µF, and 1,000 Ω
- **96 well/ Sqr:** Square wave, 250 V, 2,000 µF, 1,000 Ω, and 20 ms
- **24 well / Sqr:** Square wave, 250 V, 2,000 µF, 1,000 Ω, and 20 ms
- **12 well/ Sqr:** Square wave, 250 V, 2,000 µF, 1,000 Ω, and 20 ms

# Well Set Protocols

Well set protocols include parameters that vary in each well of a 96-well plate. The protocol includes the following well sets.

## 96 well/ Exp,Vgrad/ Cgrad

Table 10 shows the parameters that vary in each well of a 96-well plate. The protocol includes the following well sets:

- **Well sets ABCD 1–12:** Exponential wave, voltage gradient (ΔV), 350 µF, 1000 Ω
- **Well sets EFGH 1-12:** Exponential wave, current gradient (ΔC), 250 V, 1000 Ω

**Table 11. 96well/ Exp, Vgrad/Cgrad conditions that vary across the plate.**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | ΔV (V) | 100 | 100 | 100 | 200 | 200 | 200 | 300 | 300 | 300 | 400 | 400 | 400 |
| B | | | | | | | | | | | | | |
| C | | | | | | | | | | | | | |
| D | | | | | | | | | | | | | |
| E | ΔC (µF) | 200 | 200 | 200 | 350 | 350 | 350 | 500 | 500 | 500 | 1000 | 1000 | 1000 |
| F | | | | | | | | | | | | | |
| G | | | | | | | | | | | | | |
| H | | | | | | | | | | | | | |

## 96 well/ Sqr,Vgrad, Dgrad

Table 10 shows the parameters that vary in each well set of a 96-well plate. The protocol includes the following well sets:

- **Well sets ABCD 1–12:** Square wave, voltage gradient (ΔV), 2000 µF, 1000 Ω, 20 ms
- **Well sets EFGH 1-12:** Square wave, pulse duration gradient (ΔD) , 250 V, 2000 µF, 1000 Ω

**Table 12. 96 well/ Sqr,VgradDgrad conditions that vary across the plate.**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | ΔV (V) | 100 | 100 | 100 | 200 | 200 | 200 | 300 | 300 | 300 | 400 | 400 | 400 |
| B | | | | | | | | | | | | | |
| C | | | | | | | | | | | | | |
| D | | | | | | | | | | | | | |
| E | ΔD (ms) | 10 | 10 | 10 | 15 | 15 | 15 | 20 | 20 | 20 | 30 | 30 | 30 |
| F | | | | | | | | | | | | | |
| G | | | | | | | | | | | | | |
| H | | | | | | | | | | | | | |

# Mixed Protocols

The following protocols include a mix of protocols with a variety of well sets, waveforms, and plate sizes.

## Opt 96 well/ Exp,Sqr

Table 13 shows the parameters that vary in well sets for a 96-well plate. The protocol varies in a wide range of starting conditions for exponential and square waveforms.

**Table 13. Opt 96 well/ Exp, Sqr conditions that vary across the plate.**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EXP | A | 150 V 350 µF | 200 V 350 µF | 250 V 350 µF | 300 V 350 µF | 350 V 350 µF | 450 V 350 µF | 250 V 200 µF | 250 V 250 µF | 250 V 350 µF | 250 V 500 µF | 250 V 750 µF | 250 V 1000 µF |
| | B | | | | | | | | | | | | |
| | C | | | | | | | | | | | | |
| | D | | | | | | | | | | | | |
| SQR | E | 150 V 20 ms | 200 V 20 ms | 250 V 20 ms | 300 V 20 ms | 350 V 20 ms | 450 V 20 ms | 250 V 5 ms | 250 V 10 ms | 250 V 15 ms | 250 V 20 ms | 250 V 25 ms | 250 V 30 ms |
| | F | | | | | | | | | | | | |
| | G | | | | | | | | | | | | |
| | H | | | | | | | | | | | | |

## Opt 24 well/ Exp,Sqr

These are parameters that vary in each well for a 24-well plate. Except for the larger well size, the protocol is the same as “Opt 96 well/ Exp,Sqr” on page 38.

## Opt 12 well/ Exp,Sqr

Table 14 shows the parameters that vary in each well for a 12-well plate. The protocol varies in a wide range of starting condition for exponential and square waveforms in well sets.

**Table 14. Opt 12 well/ Exp, Sqr conditions that vary across the plate.**

| | Exponential wave | | | | | | Square wave | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | 200 V 350 µF | 300 V 350 µF | 400 V 350 µF | 250 V 200 µF | 250 V 350 µF | 250 V 500 µF | 200 V 20 ms | 250 V 20 ms | 300 V 20 ms | 250 V 15 ms | 250 V 20 ms | 250 V 25 ms |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

## Uniform 96 well/ Exp, Sqr

Table 15 shows the parameters that vary in each well for a 96-well plate. The protocol varies the starting conditions for exponential and square waveforms in well sets:

- **Well sets ABCD 1-6 and EFGH 1-6:** Exponential, 250 V, 350 µF, 1000 Ω
- **Well sets ABCD 7-12 and EFGH 7-12:** Square wave, 250 V, 20 ms, 1000 µF, 1000 Ω

**Table 15. Uniform 96 well/ Exp conditions that vary across the plate.**

| | Exponential wave | | | | | | Square wave | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | 250 V 350 µF | | | | | | 250 V 20ms | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

## Uniform 24 well/ Exp,Sqr

These are parameters that vary in each well for a 24-well plate. Except for the larger well size, the protocol is the same as “Uniform 96 well/ Exp, Sqr” on page 39.

## Uniform 12 well/ Exp,Sqr

These are parameters that vary in each well for a 12-well plate. Except for the larger well size, the protocol is the same as “Uniform 96 well/ Exp, Sqr” on page 39.Mixed 96 well/ Exp,Sqr

Table 16 shows the parameters that vary in well sets for a 96-well plate. The protocol varies in a wide range of starting condition for exponential and square waveforms in well sets with alternating rows of exponential (250 V, 350 µF) and square waves (250 V, 20 ms).

**Table 16. Mixed 96well/Exp, Sqr conditions that vary across the plate.**

| | Exp | Sqr | Exp | Sqr | Exp | Sqr | Exp | Sqr | Exp | Sqr | Exp | Sqr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 250 V 350 µF | 250 V 20ms | 250 V 350 µF | 250 V 20 ms | 250 V 350 µF | 250 V 20 ms | 250 V 350 µF | 250 V 20 ms | 250 V 350 µF | 250 V 20 ms | 250 V 350 µF | 250 V 20 ms |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

## Mixed 24 well/ Exp,Sqr

These are parameters that vary in each well for a 24-well plate. Except for the larger plate size, the protocol is the same as “These are parameters that vary in each well for a 12-well plate. Except for the larger well size, the protocol is the same as “Uniform 96 well/ Exp, Sqr” on page 39.Mixed 96 well/ Exp,Sqr” on page 40.

## Mixed 12 well/ Exp,Sqr

These are parameters that vary in each well for a 12-well plate. Except for the larger plate size, the protocol is the same as “These are parameters that vary in each well for a 12-well plate. Except for the larger well size, the protocol is the same as “Uniform 96 well/ Exp, Sqr” on page 39.Mixed 96 well/ Exp,Sqr” on page 40.

# 6 Factors Affecting Electroporation

The Gene Pulser MXcell™ electroporation system can help you design successful electroporation experiments. Read the sections in this chapter for information that can assist you in designing your electroporation experiments:

- Factors affecting electroporation (page 41)
- Electroporation Theory (page 45)

## Factors Affecting Electroporation

When considering electroporation of an unfamiliar cell line, we generally recommend reviewing the protocols from several references and making a consensus starting protocol. If no references exist for your particular cell line of interest, then we suggest using references for a similar cell type as a starting point. Alternatively, select the Opt mini 96-well/ Sqr, Exp pre-set protocol (page 34) to find initial conditions, then fine tune the conditions.

The following sections discuss different factors that affect the success of an electroporation experiment:

- Waveform (page 41)
- Duration and number of pulses (page 42)
- Cell growth (page 42)
- Nucleic acids and biomolecules (page 42)
- Electroporation buffer (page 43)
- Temperature (page 44)

### Waveforms

The two most common waveforms used in electroporation today are the square wave and exponential. The square wave relies on a charge being applied to the cells for a set time. The exponential waveform builds up a charge in a capacitor, and when applied to the sample, the voltage delivered decays exponentially, until the charge remaining is about 37% of the original pulse. The time over which this decay occurs is known as the time constant and is equal to (RxC) where the resistance of the sample and system is R and the capacitance set on the instrument is C.

## Time Constant and Number of Pulses

While square waves do not report a time constant, they instead are determined by the pulse duration (or pulse length), which is a time in milliseconds that is programmed into the instrument being used. It is possible to use shorter or longer pulse durations when optimizing square wave electroporation. Generally small increments are used. You may want to test 5 msec lower and higher than the original pulse duration Additionally, it has been observed that with square wave electroporation, cells might benefit from multiple, shorter pulses. For example, if the optimal pulse duration is 20 msec, it may be possible to further optimize by giving two pulses of 10 msec duration each.

The time constant in exponential waveforms is directly related to the resistance of the sample and the resistance setting used on the electroporator, as well as the capacitance setting on the electroporator. Resistance of the sample can be affected by either changing the sample volume or using an electroporation buffer with a higher or lower ionic strength. Decreasing the sample volume leads to an approximately proportional increase in sample resistance, thus nearly doubling the time constant.

## Cell Growth

For mammalian cells, the highest expression following electroporation is obtained when cells are in mid-log phase growth (Anderson et al.,1991). Healthy cells transfect better than poorly maintained cells. Routinely subculturing cells before they become overcrowded or unhealthy will minimize experimental variability in continuous cell lines. Since cells may gradually change in culture, using cells within a defined passage number and adhering to strict protocols, including parameters for intervals between plating and transfecting cells, will improve experimental reproducibility. It is also important that the cells be healthy and not contaminated with mycoplasma.

The highest gene expression following electroporation is obtained using cells which are actively growing and dividing rather than in stationary growth phase. For optimum cell recovery, the cell density in each well should be in the range of $10^6$-$10^7$ cells/ml; higher cell concentrations may result in undesirable cell fusion.

## Nucleic Acid and Biomolecules

The transfection efficiency of electroporation can be affected by the concentration, the purity, and size of the molecules used.

While the majority of electroporation applications involve delivery of plasmid DNA and siRNAs to cells, nearly any type of molecule can be introduced into cells by electroporation, including RNA, proteins, carbohydrates, and small molecules, such as nucleoside triphosphates and fluorescent dyes.

### DNA

With few exceptions, when delivering autonomously replicating plasmids, the highest transformation efficiencies are obtained when electroporating supercoiled plasmid. However, integration of electroporated plasmid into the host genome is usually most efficient using linearized plasmid, such as when isolating stable transformants of mammalian cells (Barsoum 1995), Addition of a carrier, such as salmon sperm DNA or plasmid, has also been shown to increase gene expression in some cell lines (Chu, et al.1987; Showe, et al. 1990).

Although transformation of most cell types has been accomplished using plasmid DNA isolated by a variety of methods, the sample purity has an effect on transformation efficiency. Significantly lower transformation efficiencies are generated with unpurified plasmid DNA than with purified plasmid DNA. Plasmid produced using the Bio-Rad Aurum matrix is as efficient as CsCl-purified plasmid for transformation of mammalian cells. However, as long as plasmids used for electroporation are all prepared in the same manner, changes in expression levels are due to differences in transcription or translation of the gene of interest. The concentration range for plasmid DNA electroporation is typically 5 to 40 µg/ml, and is dependent of the cell type used.

### siRNA

The quality of siRNAs can significantly influence the outcome of siRNA transfections and RNAi experiments. The siRNAs should be free of reagents carried over from synthesis. Also, dsRNA contaminants larger than approximately 30 bp cause cytotoxicity. In addition, undesired off-target effects can be avoided by using highly purified siRNAs.

The optimal siRNA concentration and its capacity for gene silencing are influenced in part by properties of the target gene including the following: mRNA localization, stability, and abundance; and target protein stability and abundance. If too much siRNA is used in electroporation, it may be toxic. Conversely, if too little siRNA is transfected, reduction of target gene expression may be undetectable. The optimal amount must be determined empirically by varying the siRNA amount within a limited range. We recommend using siRNAs at concentrations of 10 to 100 nM.

Experiments involving siRNAs have been mostly limited to immortalized cell lines, because these cells are relatively easy to grow, maintain, and transfect. However, primary cells are often a preferable model for studying gene function because they are more similar to their in vivo counterparts than immortalized cell lines. Electroporation provides a highly efficient method for direct transfer of siRNAs into primary cells.

Impurities in siRNA oligonucleotide preparations can reduce the potency and delivery efficiency of siRNAs, and can increase the risk of toxicity in gene silencing experiments. Using high quality duplex siRNA, siLentMer™ Dicer-Substrate siRNA duplexes will lead to improved success rates and reproducibility of gene silencing experiments.

## Electroporation Buffer

The electroporation medium influences cell viability and transfection efficiency in several ways. The osmotic balance of the electroporation buffer, the choice of salt, and the requirements of specific ions all play a role in electroporation. In general, a media that will mimic the natural cytoplasmic composition of the cell, such as Gene Pulser electroporation buffer is recommended.

The buffer used to electroporate mammalian cells has a direct effect on the time constant, since the sample resistance (R) is mainly due to the buffer ionic strength. The buffer components also influence transfection efficiency and cell viability. Traditionally, a buffer with high ionic strength (low resistance) such as PBS is used when electroporating mammalian cells at a high capacitance. Serum-free growth medium has also been routinely used in electroporation. The volume of liquid/buffer in the electroporation well has a significant effect on sample resistance, and is inversely proportional to the volume of the buffer used.

Gene Pulser electroporation buffer is universal and can be used with most cell lines including primary cells. The buffer works well with both siRNA and plasmid DNA, and contains components that enhance transfection efficiency and maintain overall cell health and viability.

Gene Pulser Electroporation Buffer is lower in ionic strength than PBS (it has higher resistance), thus adjustments need to be made when switching from a protocol using a traditional low resistance buffer. The recommended starting point from which to optimize is to decrease the specified capacitance by 50%. Alternatively, the resistance setting on the electroporator may be reduced to 20% of the original protocol, while maintaining all other parameters constant.

## Temperature

Electroporation of some cell types has been reported to be more efficient at 0-4°C, in other cases, room temperatures yields better results. Temperature may affect physical properties of the membrane, and influences the rate of the duration of the permeabilized state. Electroporated cells may remain permeable for several hours if low temperatures are maintained. Loss of permeability is retarded when cells are maintained at 0°C, but occurs within a matter of minutes when cells electroporated at 0°C are subsequently incubated at 37°C.

The temperature at which cells are maintained during electroporation is expected to have a role in the efficiency of the electroporation process for several reasons:

- Since passing an electric pulse through the cells results in heating, keeping the cells at a low temperature during the pulse might reduce heating and therefore increase cell viability
- Electroporation involves the transient formation of pores in the cell membrane, keeping cells at a low temperature after the pulse might allow the pores to remain open longer, giving the DNA in the medium more time to enter the cells. Alternatively, a higher temperature may speed pore closure and increase cell viability
- Changing the temperature of a solution changes its conductivity. The conductivity of the medium increases with increasing temperature, resulting in a decrease in the medium resistance and a decrease in the time constant
- Diffusion rate is highly dependent on temperature therefore keeping cells at a low temperature would be expected to reduce the diffusion of molecules across the cell membrane. In practice, the most efficient temperature at which to electroporate cells must be determined empirically

For most mammalian cells, electroporation is most efficient when cells are maintained at room temperature before and after the pulse (Chu, et al. 1987), although some cell types are more efficiently transformed at low temperature (Potter, et al. 1984).

NOTE: When using Gene Pulser electroporation buffer, electroporation should be performed at room temperature.

# Electroporation Theory

Electroporation is a physical process in which cells are exposed to a high-voltage electric field resulting in a temporary rearrangement of the cell membrane. As a result, the cells become permeable and may take up solutes from their surrounding environment, including nucleic acids, proteins, carbohydrates, and small molecules. While much work has been done to determine how cells become permeabilized during the process of electroporation, the membrane changes that occur are still largely hypothetical (Chang et al., 1992).

There are two instrument parameters that describe the changes that cells experience upon electroporation. The first of these, the electric field strength, E, measured in V/cm, describes the electrical environment in the electroporation chamber (plate chamber). Standard electrodes used in electroporation consist of two parallel plates separated by a distance d (cm); therefore, E = V / d, where V is the applied voltage and d is the distance between the electrodes. In practical terms, the field strength is manipulated by altering the voltage of the instrument or by changing the distance between the electrodes. Because the electric conductance of the cell cytoplasm is much higher than that of the cell membrane, placing the cell in an electric field creates a voltage potential across the cell membrane. As the field strength increases, the transmembrane voltage experienced by the cell increases, as does the likelihood that a pore will form in the membrane due to breakdown of the lipid bilayer, allowing molecules to enter the cell from the outside (Hui 1995; Neumann, et al. 2000).

The second parameter that affects the cell membrane is the length of time that it is exposed to the electric field. For exponential decay pulses, this is controlled by the capacitance of the instrument and the resistance within the circuit. For square wave pulses the pulse length is controlled directly by setting the time that the cells are exposed to the electric field. These are discussed for each pulse type below.

The Gene Pulser MXcell system is the only electroporation instrument capable of delivering both exponential decay (page 45) and square wave pulses (page 46) with different protocols to 24 well sets in a single plate. The system consists of a pulse generator system (the power module), a plate chamber and electroporation plate with incorporated electrodes (page 5). Activating the PULSE button on the Gene Pulser MXcell system charges the capacitors in the unit to a high voltage. Then the system causes current flow from the capacitor into the sample in the electroporation plate. Discharging the charged capacitor into the sample generates either the exponential decay or the square wave pulse.

## Exponential Decay Pulses

The exponential decay circuit of the Gene Pulser MXcell electroporation system generates an electrical pulse by discharging a capacitor. When a capacitor is discharged into the sample, the voltage across the electrodes rises rapidly to the peak voltage then declines over time t, with an exponential decay waveform (Figure 11 on page 47) according to the following equation:

$$V_t = V_o^{[e\ -(t/RC)]}$$

where $V_o$ is the initial voltage in the capacitor, $V_t$ is the voltage at time = t (expressed in ms) after the pulse, e is the base of the natural logarithm, R is the resistance of the circuit (expressed in Ω), and C is the capacitance (expressed in µF). The time required for the initial voltage to drop to $V_o/e$ is referred to as the time constant, $T$, a convenient expression of the pulse length (expressed in msec). When t = $T$ = R x C, the voltage has declined to 1/e (~37%) of the initial value, $V_o$ ($V_T = V_o / e$).

By changing the capacitor of the instrument or by changing the resistance of the circuit, the time constant may be readily changed. For resistors connected in parallel, the total resistance of the circuit is given by the following equation:

$R_T = (R_{sample} * R_{PC}) / (R_{sample} + R_{PC})$

When the sample resistance is much greater than the parallel resistor in the PC module ($R_{sample} >> R_{PC}$), the latter is the primary determinant of the resistance of the circuit, and $R_T \cong R_{PC}$. Therefore, the parallel resistance reduces the resistance of the circuit thereby reducing the time constant of the circuit.

When low-resistance media are used (e.g., high ionic-strength media such as PBS or growth media used for most mammalian cells), the time constant is most easily manipulated by selecting the proper capacitor in the Gene Pulser MXcell system. Additionally, changing the volume of low-resistance media in the cuvette will alter the resistance of the circuit (resistance is inversely proportional to volume).

## Square Wave Pulses

Truncating the pulse from a capacitor after discharging it into the sample generates square wave pulses. The ideal square wave pulse has the same voltage at the end as at the beginning of the pulse (Figure 11 on page 47). However, when using a charged capacitor to produce this waveform (as is done in all commercially available electroporation instruments), the voltage at the end of the pulse, $V_t$, is always less than the voltage at the beginning of the pulse, $V_o$. This is because when the switch is closed across a charged capacitor, maximum current instantaneously flows through the circuit and gradually falls to zero. To produce a square wave, the pulse is terminated at some time t, following discharge of the capacitor. This time (t) is termed the pulse length. The longer the pulse length, the greater is the difference in voltage between the beginning and the end of the pulse. This voltage decay may be determined from the following equation:

$\ln (V_o / V_t) = t / (R\ C)$

The decrease in voltage that occurs with a square wave pulse is inversely related to both the capacitance of the instrument and the resistance of the sample. The decrease in voltage at the end of the pulse is termed droop. The fractional decrease in voltage is determined by the following equation:

Fractional Voltage Decrease (% droop) = $(V_o - V_t) / V_o$

Combining two equations the previous two equations results in the following equation:

$\ln [1 / (1 - \%\ \text{droop})] = t / (R\ C)$

In order for the pulse to most closely approximate a true square wave, droop must be minimized (i.e., $V_t = V_o$ and $V_o - V_t = 0$). Experimentally, this is achieved by choosing the highest values for R and C. For any given sample, R may be considered a constant. For each selected protocol, C may also be considered a constant. Therefore, for the same sample, as pulse length increases, droop also increases. However, increasing sample resistance reduces the droop at any given pulse length. Increasing the sample resistance may be accomplished by the following conditions:

- Reducing the temperature of the sample
- Reducing the ionic concentration of the solution
- Reducing the volume of liquid in the electroporation cuvette in the case of low-resistance media.

Table 17 lists the fractional voltage decrease (% droop) associated with pulse length at various sample resistances for the high-voltage and low-voltage ranges on the Gene Pulser MXcell system. For example, pulsing into a 200-Ω load, the pulse length will be 33.4 msec with a 5% fractional voltage decrease in the low-voltage range.

**Table 17. Fractional voltage decrease (% droop) associated with pulse length.**

| **Low-Voltage Circuit** | | | |
|---|---|---|---|
| Fractional voltage decrease (% droop) | 10 | 20 | 5 |
| Sample | | | |
| Resistance (Ω) | | Pulse length (ms) | |
| 20 | 3.34 | 7.14 | 14.6 |
| 200 | 33.4 | 71.4 | 146 |
| 1000 | 167 | 357 | 730 |
| 3500 | 585 | 1249 | 2556 |

Figure 11 shows an exponential decay pulse from a capacitance discharge system. When a capacitor, charged to an initial voltage $V_o$, is discharged into cells, the voltage applied to the cells decreases over time in an exponential curve such that the voltage V at any given time t is given by $V = V_o\, e^{-(t/RC)}$. In the special case where t = CR then $V_o/e$ the value CR is known as the time constant of the voltage decay. The shorter the time constant the faster the decay.

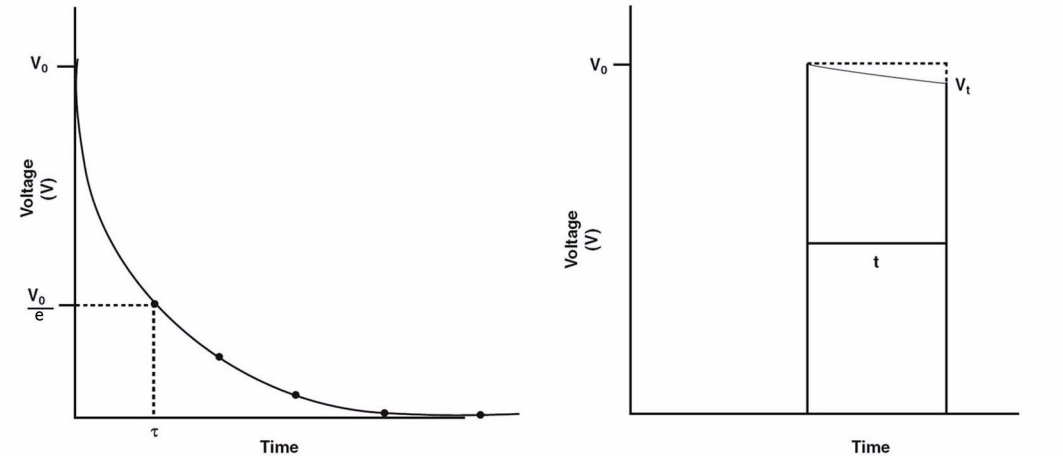



**Figure 11. Exponential decay pulse and square wave pulse.**

Figure 11 also shows a square wave pulse from a capacitance discharge system. The pulse length is the time the cells are subjected to the discharge. During the pulse the voltage again decays in an exponentially so that at the end of the pulse the voltage is lower than at the beginning. We call this drop in voltage the pulse “droop” and measure it as a percentage of the initial voltage.

## Resistor Pulse Modulation

The Gene Pulser MXcell electroporation system uses a new system for controlling parallel resistance. This new system incorporates the function provided by the Gene Pulser MXcell CE Module, but within the smaller space available in the Gene Pulser MXcell power module. The new system (called RPM for “resistor pulse modulation”) varies at high frequency the time a parallel resistance is connected to the output of the Gene Pulser MXcell system. By changing the duty cycle of this connection time, the effective value of the parallel resistance can be varied. The selected Gene Pulser MXcell system capacitance averages the resulting pulse-modulated resistance. This approach requires fewer components, uses less space, provides a wider resistance range (50-1,500 Ω), and increases flexibility of the Gene Pulser MXcell system.

# Appendix A: PulseTrac™ System

The Gene Pulser MXcell™ electroporation system uses the PulseTrac waveform delivery system to generate the most accurate exponential decay pulses possible for optimal cell transformation. PulseTrac accurately calculates the time constant and amplitude of each pulse based on what is actually delivered to the sample. More importantly, the PulseTrac system compensates for amplitude changes caused by varying sample resistances and improves the accuracy of the low voltage capacitors. This provides:

- Sample conductivity measurement integrated with pulse output for true waveform delivery for samples with resistance in the 10–1000 ohm range
- Internal calibration and circuit monitoring program for accurate pulse delivery throughout the lifetime of the unit
- Time constant and voltage amplitude measurements to allow pulse delivery verification

The PulseTrac test algorithm is activated upon startup of the Gene Pulser MXcell system.

## PulseTrac System Description

The PulseTrac system monitors and adjusts for the internal system resistance used to limit current and the sample resistance in the well. Sample resistance depends on its conductivity, the distance between the electrodes in the well, and the volume of media within the well. PulseTrac circuitry monitors the resistance of the sample and delivers the desired voltage regardless of sample volume or conductivity. When you are optimizing electroporation with the PulseTrac system, the electrical variables are controlled with exacting precision so that your results reflect only the biological variables in your experimental design. This PulseTrac diagnostic algorithm examines the complete electrical circuit and electronically selects the right combination of capacitors to deliver the most accurate and reproducible pulse for optimal and consistent electroporation over the lifetime of the unit.

# PulseTrac Diagnostic Algorithm

The PulseTrac diagnostic algorithm tests and selects the optimal capacitor circuit of the Gene Pulser MXcell power module in the range of 25–2,475 µF. This is the key bank of capacitors used in low-voltage/high-capacitance precision pulse delivery. The diagnostic algorithm tightens the already rigorous capacitor tolerance from ±20% to ±10% (other unit designs can have a capacitor variance as high as ±40%) in the 200–1,075 µF range. The high voltage capacitors in the Gene Pulser MXcell unit are not part of this system, they are preselected to the same 10% tolerance that the diagnostic algorithm provides

# Appendix B: Troubleshooting

Follow these troubleshooting options:

**1. Little or no transfection or cell viability.**

- **Check well sets.** All wells in a well set must be filled with either sample or sample buffer. For example, if you want to electroporate six wells, fill a complete well set (such as ABCD1) with sample and fill two wells in a second well set (such as AB2) with sample. Finally, be sure to fill the remaining two wells in the second well set (such as CD2) with the sample buffer.
- **Check capacitance (for Square wave form protocol).** A square waveform may require higher capacitance values. Use 2000 µf if you are experiencing no transfection when using a square wave form protocol.
- **Check sample volume.** If you are working with the lower limit such as 100 µl in a 96-well plate, increase the sample volume. Increasing sample volume will tend to increase cell viability and transfection efficiency.

**2. Gene Pulser MXcell™ electroporation system stalls or hangs.**

- **Check sample volume.** If your wells contain too little volume the Gene Pulser MXcell electroporation system will not detect an arc. The instrument will stall and will not produce a report. In this case, the Gene Pulser MXcell electroporator must be reset by turning the unit off and on again.

  NOTE: Before resetting the unit, determine at which well set the instrument stalled. Failure to do so will result in loss of the data report once the Gene Pulser MXcell is reset. All wells before the stall occurred should be correctly electroporated, and all wells after the stall are not correctly electroporated.
- **Check your settings.** Arcing can occur when using upper limit settings. For example, 3 pulses and duration (>20 ms) is one upper limit.

# Appendix C: References

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# Appendix D: Product Specifications and Information

The following tables provide product specifications and information about the Gene Pulser MXcell™ electroporation system, including operating specifications and catalog numbers.

## Product Specifications

Table 18 lists specifications for the entire system, including the power module and plate chamber:

**Table 18. System specifications for Gene Pulser MXcell electroporation system**

| **Waveform** | **Exponential decay or Square wave** |
|---|---|
| Voltage | 10–500 V in 2 V increments |
| Capacitance | 25–2475 µF in 25 µF increments |
| Resistance (Parallel) | 50–1,500 Ω in 50 Ω increments plus infinity |
| Sample Resistance | 10 Ω minimum at 10–500 V; 125 Ω with Gene Pulser electroporation buffer |
| Square Wave Timing | 10–500 V: 0.05–9999.95 ms pulse length, 1–3 pulses |
| | 0.1–10 sec pulse interval |

Table 19 lists the general specifications for the entire system:

**Table 19. General specifications for Gene Pulser MXcell electroporation system**

| **Type** | **Specification** |
|---|---|
| Input Voltage | 100–120 VAC or 220–240 VAC, 50/60 Hz |
| Power | Max 240 W (During short charging periods) |
| Operating environment | Temperature 0–35°C, Humidity 0–95%, (non-condensing) |
| Regulatory | Safety EN 61010, EMC EN61326 Class A |
| Dimensions | Power Module 31 x 30 x 14 cm, weight 6.62 kg |

# Product Information

Table 20 lists catalog numbers and descriptions for the system and accessories:

**Table 20. Catalog numbers for Gene Pulse MXcell system**

| Catalogue no. | Description |
|---|---|
| 165-2670 | Gene Pulser MXcell electroporation system100–240 V, 50/60 Hz, exponential decay and square wave delivery, includes power module, plate chamber, and 1 x 96-well electroporation plate, instruction manuals |
| 165-2671 | MXcell Power Module |
| 165-2672 | Plate Chamber |
| 165-2681 | 1 x 96-well Electroporation Plate |
| 165-2682 | 1 x 24-well Electroporation Plate |
| 165-2683 | 1 x 12-well Electroporation Plate |
| 165-2094 | Gene Pulser Electroprotocols |
| 165-2676 | Gene Pulser Electroporation Buffer, 1.8 ml x 10 |
| 165-2677 | Gene Pulser Electroporation Buffer, 30 ml |

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