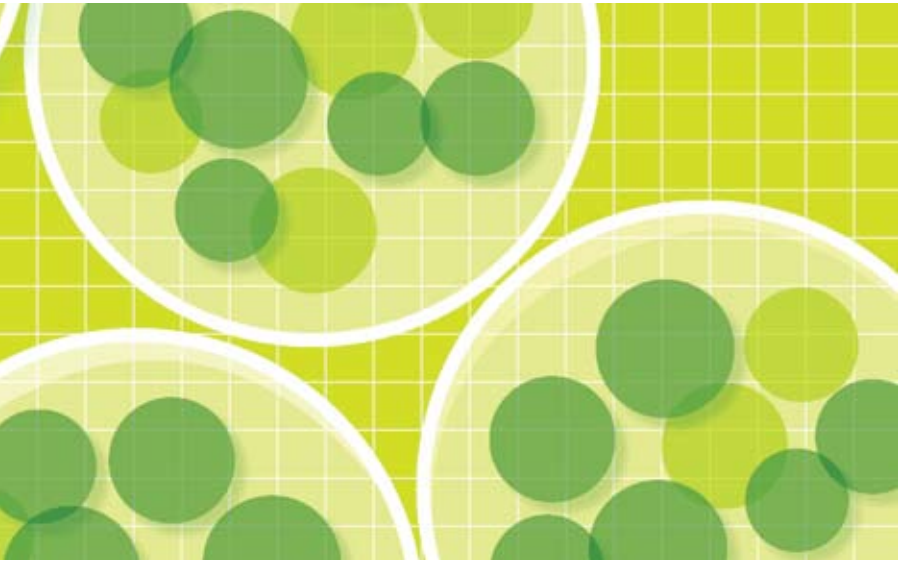


Gene Pulser MXcell™

Electroporation Guide

BIO-RAD

The Gene Pulser MXcell Electroporation Guide

The Gene Pulser MXcell electroporation 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. This guide will assist you in determining the parameters for obtaining efficient transfection of molecules into your favorite cells while maintaining high cell viability.

Transfection efficiency and cell viability

Transfection and cell viability are affected by:

- Cell type and its physiological condition and state prior to electroporation: cells should be actively growing, healthy, and free of contamination
- Temperature of buffer during electroporation: when using Gene Pulser® electroporation buffer, electroporation should be performed at room temperature
- Cell density: for most experiments using the 96-well plate, we recommend using 150 µl of cells/well at a density of 1 x 10^6 to 2 x 10^6 cells/ml in Gene Pulser electroporation buffer
- Concentration of the transfected molecule (RNA, DNA): ideally a concentration of 5–40 µg/ml should be used for plasmid DNA and 10–100 nM for siRNA (only Bio-Rad's siLentMer™ Dicer-substrate siRNA duplexes)
- The characteristics of the electric pulse

To choose a starting set of protocols, search our library of electroprotocols at www.bio-rad.com/genetransferprotocols/ or search http://www.ncbi.nlm.nih.gov/PubMed/.

Electroporation protocols

Electroporation applies an electric pulse to cells to promote uptake of molecules (RNA, DNA) into the cells. The electric pulse is defined by parameters that can be programmed as protocols in the Gene Pulser MXcell. Two type of pulses can be delivered by the Gene Pulser MXcell: **the exponential waveform** which is defined by **voltage, capacitance, and resistance** or **the square waveform** which is defined by **voltage, pulse duration, and resistance**.

The Gene Pulser MXcell can be programmed three ways:

- Manually, under "Protocol Setup"
- By creating a voltage gradient under "Gradient Protocol"
- By using preprogrammed protocols under "Pre-Set Protocols"*

This guide will help you program the Gene Pulser MXcell whether you have prior knowledge of optimal electroporation conditions for your cells or not.

*** Note: The parameters entered into the preset protocols are based on proven conditions used for electroporation of mammalian cells and take advantage of the Gene Pulser MXcell's ability to apply multiple conditions simultaneously. The preset protocols can be edited and stored in your user file for future use.**

Glossary

Electroporation plate: a plate in which electroporation is performed; available in 12-, 24-, and 96-well formats

Mini protocol: a preprogrammed set of 4–6 different protocols delivered to 4–6 well sets

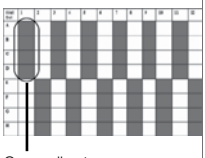
Parameters: the physical constants (waveform, voltage, capacitance, duration, resistance) that define the electric pulse

Preset protocols: a set of preprogrammed protocols designed for rapid screening of multiple parameters that can be used as a template

Protocol/electroporation conditions: parameters defining the electric pulse that will be delivered to specific well sets or wells on an electroporation plate

Waveform: defines the type of electric pulse delivered to the cells

- The exponential waveform builds up a charge in a capacitor and when the charge is applied to the sample the voltage delivered decays exponentially, until the charge remaining is about 37% of the original pulse
- The square waveform relies on a charge being applied to the cells for a set time



One well set

Schematic of 96-well electroporation plate

Well set: a set of four adjacent wells in a column on a 96-well plate; the same electroporation conditions or protocol are applied to all the cells of a well set

Whole plate protocol: a preprogrammed set of 24 protocols delivered across the entire plate

Abbreviations

Cgrad: capacitance gradient

D: duration of pulse

Dgrad: duration gradient

Exp: exponential

NP: number of pulses

P: pulse

Sqr: square

Vgrad: voltage gradient

Electroporation Parameters Selection Pathways

The first parameter to identify is the waveform. This guide will take you down two possible paths to identify other parameters that will yield the best transfection results. These parameters can be identified in parallel using the MXcell system.

Identify Pulse Waveform

Exponential Waveform	Square Waveform
Optimize Capacitance	Optimize Voltage
Optimize Voltage	Optimize Pulse Duration
Optimize Resistance	Optimize Number of Pulses

Path taken to determine optimal electroporation conditions

Start Your Protocols Selection Here

To choose a starting set of protocols, search our library of electroprotocols at www.bio-rad.com/genetransferprotocols/ or search http://www.ncbi.nlm.nih.gov/PubMed/.

Known electroporation conditions

You know the electroporation conditions or found a suitable set of protocols in our library

You need a quick confirmation that your existing protocol is optimal

You know the waveform

Go to page 6

Unknown electroporation conditions

You are working with a new cell line or cell type

You have no information about electroporation conditions

Go to page 7

Known Electroporation Conditions

Cell number
Based on the number of cells available for your experiment, you will use either a whole or a partial electroporation plate (4–6 well sets).

Cell number <5 x 10^6
Use Mini Protocols

Go to pages 9–10

Cell number >5 x 10^6
Use Whole Plate Protocols

Go to pages 11–12

Multiple cell lines
Use Whole Plate Protocols

Go to page 13

Unknown Electroporation Conditions

Cell number
Based on the number of cells available for your experiment, you will use either a whole or a partial electroporation plate (4–6 well sets).

Cell number <5 x 10^6
Use Mini Protocols

Go to page 8

Cell number >5 x 10^6
Use Whole Plate Protocols

Go to page 8

Unknown Electroporation Conditions

Check all well sets after each electroporation for transfection efficiency and cell viability.

For an overview of common analytical methods, go to pages 15–17.

Cell number <5 x 10^6

Use preset protocols **Opt mini 96-well/Sqr, Exp** to identify waveforms and other initial conditions.

Good for first time users!

	Square waveform			Exponential waveform		
	1	2	3	4	5	6
A	200 V 2,000 µF 20 ms	250 V 2,000 µF 20 ms	300 V 2,000 µF 20 ms	250 V 350 µF 1,000 Ω	250 V 500 µF 1,000 Ω	250 V 750 µF 1,000 Ω
B						
C						
D						

Cell number >5 x 10^6

Use preset protocols **Opt 96-well/Exp, Sqr** to identify waveforms and other initial conditions.

		1	2	3	4	5	6	7	8	9	10	11	12
Exponential	A	150 V 350 µF	200 V 350 µF	250 V 350 µF	300 V 350 µF	350 V 350 µF	400 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 1,000 µF
	B												
	C												
	D												
Square	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												

Would you like to further optimize? Use the preset protocols on pages 9–10 (if <5 x 10^6 cells) **or pages 11–12** (if >5 x 10^6 cells)**; for manual programming, go to page 14.**

Good results? Congratulations! Be rewarded for your work and share your protocol with scientists worldwide. Visit www.bio-rad.com/genetransferprotocols/.

Electroporation Conditions Are Known

Cell number <5 x 10^6

Choose the preset mini protocols that best match your known conditions and edit parameters as needed, following the instructions.

Exponential Waveform Protocols

Use preset protocols **Opt mini 96-well/Exp** to identify optimal voltage and capacitance.

	1	2	3	4	5	6
A	200 V 350 µF	250 V 350 µF	300 V 350 µF	250 V 200 µF	250 V 350 µF	250 V 500 µF
B						
C						
D						

Keep the preset values for capacitance and resistance:

- Enter the known voltage value (median voltage) in well set ABCD 2
- Enter the median voltage decreased by 50 V in well set ABCD 1
- Enter the median voltage increased by 50 V in well set ABCD 3
- Enter the median voltage value in well sets ABCD 4–6
- Save the protocols under a new name

Check all well sets after each electroporation for transfection efficiency and cell viability.

For an overview of common analytical methods, go to pages 15–17.

Would you like to further optimize? For manual programming, go to page 14.

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Electroporation Conditions Are Known

Cell number <5 x 10^6

Choose the preset mini protocols that best match your known conditions and edit parameters as needed, following the instructions.

Square Waveform Protocols

Use preset protocols **Opt mini 96-well/Sqr** to identify optimal voltage and pulse duration.

	1	2	3	4	5	6
A	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						

Keep the preset values for pulse duration, capacitance, and resistance:

- Enter the known voltage value (median voltage) in well set ABCD 2
- Enter the median voltage decreased by 50 V in well set ABCD 1
- Enter the median voltage increased by 50 V in well set ABCD 3
- Enter the median voltage value in well sets ABCD 4–6
- Save the protocols under a new name

Check all well sets after each electroporation for transfection efficiency and cell viability. For an overview of common analytical methods, go to pages 15–17.

For further optimization of your experiment, identify the optimal number of pulses.

Square Waveform Protocols

Use preset protocols **Opt 96-well/Sqr, NP, D** to identify optimal number of pulses.

	1	2	3	4
A	20 ms 1 P	15 ms 2 P	10 ms 2 P	7 ms 3 P
B				
C				
D				

Keep the preset values:

- Enter the voltage that yielded the best results from the **Opt mini 96-well/Sqr** experiment
- Save the protocols under a new name

Would you like to further optimize? For manual programming, go to page 14.

Good results? Congratulations! Be rewarded for your work and share your protocol with scientists worldwide. Visit www.bio-rad.com/genetransferprotocols/.

Electroporation Conditions Are Known

Cell number >5 x 10^6

Choose whole plate preset protocols that best match your known conditions and edit parameters as needed, following the instructions.

Exponential Waveform Protocols

Use whole plate preset protocols **96-well/Exp, Vgrad, Cgrad** to identify optimal voltage and capacitance.

		1	2	3	4	5	6	7	8	9	10	11	12
A		100	100	100	200	200	200	300	300	300	400	400	400
B	ΔV (V)												
C													
D													
E		200	200	200	350	350	350	500	500	500	1,000	1,000	1,000
F	ΔC (µF)												
G													
H													

Check all well sets after each electroporation for transfection efficiency and cell viability. **For an overview of common analytical methods, go to pages 15–17.**

Use the top half of the electroporation plate to identify optimal voltage.

- Enter the known capacitance value in well sets ABCD 1–12, or keep preset values if unknown
- Enter the known voltage (median voltage) value in well sets ABCD 4–6
- Vary the voltage by 100 V increments around the median value in other well sets
- Keep the preset values for all other parameters

Use the bottom half of the electroporation plate to identify optimal capacitance.

- Enter the known voltage value in well sets EFGH 1–12
- Enter the known capacitance (median capacitance) value in well sets EFGH 4–6
- Vary capacitance by 5–10% around the median value in other well sets or use preset values
- Keep the preset values for all other parameters

Save the protocols under a new name.

Would you like to further optimize? For manual programming, go to page 14.

Good results? Congratulations! Be rewarded for your work and share your protocol with scientists worldwide. Visit www.bio-rad.com/genetransferprotocols/.

Electroporation Conditions Are Known

Cell number >5 x 10^6

Choose whole plate preset protocols that best match your known conditions and edit parameters as needed, following the instructions.

Square Waveform Protocols

Use whole plate preset protocols **96-well/Sqr, Vgrad, Dgrad** to identify optimal voltage and pulse duration.

		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													

Check all well sets after each electroporation for transfection efficiency and cell viability. **For an overview of common analytical methods, go to pages 15–17.**

Use the top half of the electroporation plate to identify optimal voltage.

- Enter the known capacitance value in well sets ABCD 1–12, or keep preset values if unknown
- Enter the known voltage (median voltage) value in well sets ABCD 4–6
- Vary the voltage by 100 V increments around the median value in other well sets
- Keep the preset values for all other parameters

Use the bottom half of the electroporation plate to identify optimal pulse duration.

- Enter the known voltage value in well sets EFGH 1–12
- Keep the preset values for all other parameters

Save the protocols under a new name.

Would you like to further optimize? For manual programming, go to page 14.

Good results? Congratulations! Be rewarded for your work and share your protocol with scientists worldwide. Visit www.bio-rad.com/genetransferprotocols/.

Multiple Cell Lines

You work with multiple cell lines that require both waveforms.

Use whole plate preset protocols to deliver exponential and square waveforms. Edit parameters as needed, following the instructions.

Exponential and Square Waveforms Protocols

Use preset protocols **Uniform 96-well/Exp, Sqr** to identify optimal conditions for the different cell lines.

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

Use the left half of the plate to deliver exponential-decay pulses.

- Enter your known voltage (median value) and vary capacitance around the known value in well sets ABCD 1–6
- Enter your known capacitance value and vary voltage around the median voltage value in well sets EFGH 1–6
- Use the preprogrammed setting for all other values

Use the right half of the plate to deliver square-wave pulses.

- Enter your known median voltage value and vary pulse duration around the preset value in well sets ABCD 7–12
- Enter your known pulse duration or preset value and vary voltage around the median voltage value in well sets EFGH 7–12

Save the protocols under a new name.

Check all well sets after each electroporation for transfection efficiency and cell viability.

For an overview of common analytical methods, go to pages 15–17.

Would you like to further optimize? For manual programming, go to page 14.

Good results? Congratulations! Be rewarded for your work and share your protocol with scientists worldwide. Visit www.bio-rad.com/genetransferprotocols/.

Electroporation Protocols Decision Tree

Once the best waveform is identified, use this tree to program the MXcell system to improve other parameters.

Initial Experimental Results

Exponential Waveform

Optimize Capacitance

Manually program a capacitance gradient (5–10% increments) around the value that yielded the best results, keeping all other parameters constant.

Optimize Voltage

Using the optimized capacitance value, program a voltage gradient (5–10% increments) around the value that yielded the best results, keeping all other parameters constant.

Optimize Resistance

Using the optimized capacitance and voltage values, manually program a resistance gradient (5–10% increments) around the values that yielded the best results, keeping all other parameters constant.

Square Waveform

Optimize Voltage

Program a voltage gradient around the value that yielded the best results, keeping all other parameters constant.

Optimize Pulse Duration

Use the optimized voltage and keep all other parameters constant. Vary pulse values at 5 ms intervals around the pulse that yielded the best results (see protocols on page 10).

Optimize Number of Pulses

Use 2–3 shorter pulses that add up to the pulse duration that yielded the best results (see protocols on page 10).

Electroporation Protocol

Materials Needed

- Actively growing, freshly passaged mammalian cells
  - **For whole plate protocols:** 16 x 10^6 adherent cells or 48 x 10^6 cells in suspension
  - **For mini protocols (6 well sets):** 4 x 10^6 adherent cells or 12 x 10^6 cells in suspension
- Cell growth medium
- PBS
- Gene Pulser MXcell electroporation system
- 96-well electroporation plate
- Gene Pulser electroporation buffer or other buffer suitable for electroporation
- Molecule to electroporate: siRNA, such as siLentMer Dicer-substrate siRNA duplexes, or DNA
- 24-well tissue culture plates

Programming the Instrument

- Turn on the Gene Pulser MXcell
- Select "Pre-Set Protocols"
- Select appropriate protocols and edit if necessary

Setting Up the Instrument

- If the cells are adherent, trypsinize the cells and add medium to inactivate the trypsin; if the cells are in suspension, skip this step
- Pellet the cells, remove the medium, and resuspend cells in PBS by gentle pipeting; count the cells

1. **For whole plate protocols:** transfer 16 x 10^6 adherent cells or 48 x 10^6 cells in suspension to a new tube, pellet the cells, and remove PBS by aspiration.

   For mini protocols (6 well sets): transfer 4 x 10^6 adherent cells or 12 x 10^6 cells in suspension to a new tube, pellet the cells, and remove PBS by aspiration.
2. Resuspend the cells in 16 ml (4 ml for mini protocol) of Gene Pulser electroporation buffer (this provides 1x10^6 cells/ml of adherent cells or 3x10^6 cells/ml of cells in suspension).
3. Add the molecule (siRNA, DNA) to the cell suspension.
4. Pipet 150 µl of cell suspension into the appropriate wells of an electroporation plate.
5. Place the lid on the plate and gently rock the plate back and forth to wet the electrodes.
6. Place the electroporation plate securely into the plate chamber, close the lid, and press the PULSE button.
7. Transfer all or 100 µl of the cells from each electroporation well to the wells of a 24-well tissue culture plate containing 500 µl of growth medium.

   Note: using this method allows replication of the experiment, providing two duplicate 24-well tissue culture dishes.
8. Incubate cells at 37°C in a humidified CO_2 incubator until they are ready to be assayed.

Electroporation Evaluation Methods

There are many techniques available to examine cells for transfection efficiency and cell viability. We have briefly summarized three commonly used methods. It is critical to the success of your experiment to evaluate all cells that were electroporated.

Fluorescence Microscopy for Adherent Cells

Fluorescence microscopy can be used to visualize a fluorescent signal within the cell. This method is commonly used when expressing a GFP-tagged protein.

Materials

- PBS
- Fixation buffer: 2–4% formaldehyde in PBS
- 70% glycerol

Method

1. Remove the medium from the wells of the electroporation plate and wash the cells once with 500–700 µl of PBS.
2. Add 300 µl of fixation buffer to each well and incubate at room temperature for 10 min.
3. Remove the fixative, and perform 2 washes with PBS.
4. Add 70% glycerol, and store the cells at 4°C until they are ready to be analyzed by fluorescence microscopy using the appropriate filters.

Flow Cytometry

Flow cytometry can be used to measure the number of cells containing a fluorescent tag, such as a fluorescent siRNA or a GFP-tagged protein.

Materials

- Fixation buffer: 2–4% formaldehyde in PBS
- PBS
- 70% glycerol

Method

1. For adherent cells, add 100 µl trypsin per well to detach cells and add medium (200–300 µl) to inactivate the trypsin. For cells in suspension, skip this step.
2. Transfer the cell suspension to a 1.5 ml centrifuge tube and pellet the cells (300 RCF).
3. Remove the medium and resuspend the cells in 500 µl PBS.
4. Transfer the resuspended cells to a flow cytometer tube for analysis.

Electroporation Evaluation Methods, continued

Fluorometer and Scanner Analysis for Adherent Cells

Fluorometric analysis of cell lysates can be used to examine lysed cells for the presence of a fluorescence signal. This approach can be used for detecting expression of a GFP-tagged protein.

Materials

- Lysis buffer (0.5% NP-40, 10 mM Tris pH 8.0, and 1 mM EDTA)
- PBS
- 96-well dark plate with flat, clear bottom

Method

1. Remove the medium and wash the cells once with 500–700 µl of PBS.
2. Add 100 µl lysis buffer to each well and evenly distribute the lysis buffer by gently rocking the plate.
3. Incubate the plate at –80°C for 10 min.
4. Remove the plate from –80°C and allow the lysate to thaw on ice.
5. Pipet each sample 4–5 times to wash cells off the bottom of the plate.
6. Transfer the sample to a 96-well dark plate with flat, clear bottom for fluorometer or scanner analysis.

See the Gene Pulser MXcell Electroporation System manual for more information.

Additional Preset Protocols Available

Preset Protocols	Plate Type	# of Wells Used	Application	Parameters
96-well/Exp	96	96	Use for initial optimal protocol identification for many cell types	Applies the same waveform across the whole plate
96-well/Sqr	96	96	Use for initial optimal protocol identification for many cell types	Applies the same waveform across the whole plate
Mixed 96-well/Exp, Sqr	96	96	Use for mixing different waveforms by alternating rows of exponential (250 V/350 µF) and square waveforms (250 V/20 ms)	Exp: 250 V, 350 µF Sqr: 250 V, 20 ms
Mixed 24-well/Exp, Sqr	24	24	Use for mixing different waveforms by alternating rows of exponential (250 V/350 µF) and square waveforms (250 V/20 ms)	Exp: 250 V, 350 µF, 1,000 Ω Sqr: 250 V, 20 ms, 1,000 µF, 1,000 Ω
24-well/Exp	24	24	Use for initial protocol setup for many cell types	Same conditions for the whole plate
24-well/Sqr	24	24	Use for initial protocol setup for many cell types	Same conditions for the whole plate
Opt 24-well/Exp, Sqr	24	24	Use with cell line with no protocol reference; this protocol includes a range of common starting conditions	Exp: 150–450 V, 200–1,000 µF Sqr: 150–450 V, 5–30 ms
Uniform 24-well/Exp, Sqr	24	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	Exp: 250 V, 350 µF, 1,000 Ω Sqr: 250 V, 20 ms, 1,000 µF, 1,000 Ω
12-well/Exp	12	12	Use for initial protocol setup for many cell types	Same conditions for the whole plate
12- well/Sqr	12	12	Use for initial protocol setup for many cell types	Same conditions for the whole plate
Opt 12-well/Exp, Sqr	12	12	Use with cell line with no protocol reference; this protocol includes a range of common starting conditions	Exp: 150–400 V, 200–500 µF Sqr: 150–300 V, 15–25 ms
Uniform 12-well/Exp, Sqr	12	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	Exp: 250 V, 350 µF, 1,000 Ω Sqr: 250 V, 20 ms, 1,000 µF, 1,000 Ω
Mixed 12-well/Exp, Sqr	12	12	Use for mixing different waveforms by alternating rows of exponential (250 V/350 µF) and square waveforms (250 V/20 ms)	Exp: 250 V, 350 µF, 1,000 Ω Sqr: 250 V, 20 ms, 1,000 µF, 1,000 Ω

The siLentMer products are manufactured by Integrated DNA Technologies, Inc. (IDT) and are for research use only. For custom siRNA synthesis, contact IDT.

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