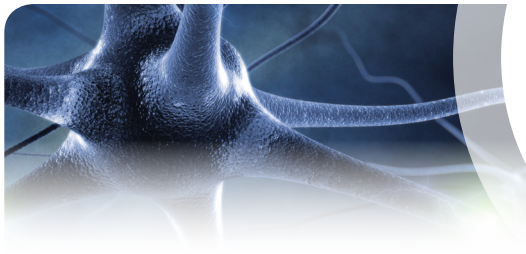

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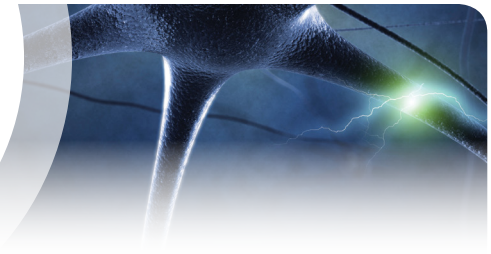

EXCLUSIVE COMPENDIUM: OPTIMIZING AN ELECTROPORATION PROTOCOL

A Harvard Bioscience Publication

Abstract
Whether you are in process of optimizing an existing electroporation method or developing an entirely new protocol, this Exclusive Compendium includes important best practice information and tips for your lab.

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Contents

Chapter 1
Converting Between Square and Exponential Decay Wave Generators

Electroporators are available with different electrical waveform styles, allowing variations of electrical pulse setting characteristics to accommodate studies of different cell types. This section highlights differences between square and exponential decay wave generators, when each is typically applied, and offers an overview of things to consider when converting from one to the other.

The two main different types of pulses used for electroporation of nucleic acids are square wave and exponential decay wave.

For both, field strength (€, usually expressed in V/cm) is dependent on the pulse parameters applied, (voltage, capacitance and resistance), and the distance between the electrode or cuvette contacts. Application of this electrical field causes Electropermeabilization (transient pores in the cell membrane through induction of transmembrane voltage) allowing nucleic acids to pass through the cell membrane.

Exponential Decay Wave

This waveform is mainly used for transforming cells with cell walls, such as bacteria and yeast, during electroporation. In this type of pulse, the set voltage is released from the capacitor—hitting peak at the beginning of the pulse. It then decays rapidly and exponentially over time (on the order of ms). The delivered pulse is characterized by two parameters: the field strength (kV/cm) and the time constant. These parameters can be adjusted by varying voltage, internal resistance and capacitance settings to achieve a wide pulse gradient.

Square Wave

During a square waveform pulse, the voltage immediately jumps to the peak voltage and is held constant for the duration of the pulse. Then it immediately drops back to zero at the end of the pulse. Square wave pulses are characterized by the voltage delivered, the duration of the pulse, the number of pulses, and the length of the interval time between pulses. This waveform is typically used for mammalian cells and tissues at a lower field strength than exponential decay wave.

Theoretical Conversion Examples

Converting from Exponential Decay Wave to Square Wave

- Select the square and set its wave pulse length to one half the time constant of the exponential decay wave protocol.
- Keep the wave voltage, the square wave gap size, pulse number, and pulse interval parameters the same as the exponential decay wave protocol.
- No adjustments are required for the Resistance (R, Ω) or Capacitance (C, μF).

Converting from Square Wave to Exponential Decay Wave

- Keep the wave voltage and the gap size parameters the same as the square wave electroporation protocol.
- Adjust the instrument resistance (R, Ω) and Capacitance (C, μF) settings on the exponential decay wave generator to achieve a time constant that is double the square wave pulse length.
- Adjust the pulse number as appropriate.

Notes: Waveform Conversion Considerations

These theoretical conversion examples are for informational purposes only. Actual conversion steps will depend on the instruments being used and cell types being studied.

Conversions may require additional optimization steps or acceptance of a performance drop when using a waveform that is not optimal for the cell type.

> "Electroporators are available with different electrical waveform styles… to accommodate studies of different cell types."

Chapter 2
Converting Between Different Gap Sizes

Optimizing your electroporation protocol requires establishment of a process to successfully convert between two electrodes or two cuvettes with different gap sizes. This section provides information to help you manage gap size conversions.

Gap Distance is defined as the distance between electrode contacts or between parallel electrode plates in a cuvette.

Electrical Field strength (expressed as Volts/centimeter or kiloVolts/centimeter) is equal to the voltage (V) set on your electroporator divided by the gap distance between your electrode contacts (cm).

Conversion Formula

The relationship between gap distance and field strength is inversely proportional; as gap distance increases, electrical field strength decreases.

To convert an electroporation protocol for different electrode gap distances, simply adjust the voltage so that the field strength (in V/cm) is constant between the two different gap sizes, while keeping the other parameters, such as pulse length and number of pulses, the same.

Example

If you normally electroporate at 500 V in a 4 mm gap cuvette, then you would want to use 250 V for a 2 mm gap cuvette, or 125 V for a 1 mm cuvette to achieve a final field strength of 1250 V/cm.

Notes: Impact of Electrode Shapes

If the electrodes are different shape, or there is a change in the relative volume of your sample, some additional fine-tuning may be necessary for best results.

“To convert an electroporation protocol for different electrode gap distances, simply adjust the voltage so that the field strength (in V/cm) is constant…”

Chapter 3
Scaling Up and Down

As part of your research, you may need to change—or scale, electroporation volumes. This section provides information to help you with scaling, including adjusting for corollary considerations that may arise when you scale.

In many cases, you can adequately scale electroporation volumes up and down by simply switching cuvette gap sizes.

- 1 mm gap cuvettes accommodate small volumes (20 to 90 µl).
- 2 mm gap cuvettes accommodate medium volumes (40 to 400 µl).
- 4 mm gap cuvettes accommodate larger volumes (80 to 800 µl).

Scaling Formula

The process of scaling up and down between different sized chambers of roughly the same dimensions (such as cuvettes) is straightforward. Simply adjust the voltage while keeping the field strength in V/cm and other parameters constant.

Example

You typically electroporate your cells for a single pulse, 5 ms, 250 V in a 2 mm gap cuvette. You want to convert to a 1 mm or 4 mm gap cuvette.

- From a 2 mm gap to a 4 mm gap cuvette, electroporate your cells for a single pulse, 5 ms, 500 V.
- From a 2 mm gap cuvette to a 1 mm gap cuvette, electroporate your cells for a single pulse, 5 ms, 125 V.

Scaling Larger Volumes

For larger volumes, you may need to move to a different type of chamber. When doing so, simply apply the previously referenced rules about adjusting voltage parameters to achieve the same field strength.

You may need to further fine-tune your protocol to account for the different geometry of the larger chamber.

Conversion Tips

For reference, the parameters normally used for a 4mm gap cuvette would also be a good starting point for a 10 ml volume, 4 mm gap large volume electroporation flatpack.

Notes: Impact to Conductance

As you increase volume, you also increase conductance of your sample, resulting in additional cell heating during the electroporation pulses which can cause cell viability issues. To address this, consider using a low conductance electroporation buffer, or experiment with a prechilled buffer for larger volume electroporations.

> “…[use] a low conductance electroporation buffer, or experiment with prechilled buffer for larger volume electroporations.”

Chapter 4
Temperature Considerations

Cell temperature can significantly affect outcomes of your experiments. Most mammalian cells can be efficiently electroporated at room temperature. However, for some research, an electroporation experiment at a lower temperature, such as 4° C, may be necessary. This section provides information to help you manage temperature considerations.

Temperature Optimization Tips

If you are not certain which temperature will work best for your cells, consider simultaneously testing several replicates—each with different temperature conditions, in a single experiment. You could include varied cell temperatures, such as room temperature, chilled to 4° C, and chilled on ice, while keeping the electroporation, transfectant, and other cell parameters constant.

Managing Cell Heating

Electroporation programs with high voltage pulses (typically used with bacteria), long pulse durations, or multiple pulses may cause increased cell heating. Similarly, by increasing volume, you may increase conductance resulting in additional heating of the sample. Because temperature increases can adversely impact cell viability, they must be considered and addressed in your protocol.

Keep Pores Open Longer

Since electroporation causes the transient formation of pores, keeping the cells at a lower temperature following the pulse may allow the pores to remain open longer to allow more uptake of the transfectant.

Pre-chill or Post-chill

To include pre-chill and post-chill steps in your protocol, simply place the cells/cuvettes on ice or in the refrigerator for a few minutes before and after electroporation. Be sure to remove any condensation on the outside of the cuvettes before placing them in the safety stand, safety dome or PEP in this process.

Temperature Adjustment Tips

The standard electroporation pulse voltage used for cells at room temperature will need to be approximately doubled for electroporation at a lower temperature (e.g., 4° C) to effectively permeate the cell membrane.

Example

If you typically electroporate cells at 250 V at room temperature, then you would want to electroporate the same cells at 500 V at 4° C.

> “Because temperature increases can adversely impact cell viability, they must be…addressed in your protocol.”

Chapter 5
Considering Transfectant Amount

Transfectant is the molecule of interest that you wish to deliver to your target cells or tissues. The amount of transfectant used in your experiment can impact transfection efficiency, as well as the results of your research. This section provides information to help you manage transfectant amounts.

Determining Optimal Transfectant Amount

Increasing the amount of transfectant can boost efficiency—up to a point. To determine the optimal amount to use in your specific research, consider running a titration experiment in advance of your work.

To do this, simply test a range of transfectant amounts, while keeping cell numbers, buffer, and pulse generator settings constant. At the conclusion of the titration experiment, you can assess the transfection efficiency and cell viability for each transfectant amount.

Since the optimal transfectant amount may vary from one construct to another, it is highly advisable to run a titration experiment each time you begin transfecting a new nucleic acid, protein, or other molecule of interest.

Typical Transfectant Ranges for Mammalian Cells

- **DNA: 5 to 20 µg per ml**
  Although sometimes increasing to higher concentrations, such as 50 to 100 µg/ml, can further enhance transfection efficiency.

- **siRNA or miRNA: 5 to 100 nM final concentration**
  The optimal siRNA or miRNA concentration will be a balance of achieving target knockdown while minimizing off-target effects.

  - You may need to adjust the concentration, depending on the potency of the particular siRNA or miRNA and the types of stabilizing modifications on the molecule.
  - Modified siRNA typically are used at a final concentration in the 5 to 20 nM range.
  - Unmodified siRNA are more commonly used at a final concentration in the 50 to 100 nM range.

- **mRNA: 1 to 10 µg per million cells**
- **Protein: 1 to 10 µg per million cells**

Transfectant Management Tip

Dilute your transfectant in sterile nuclease-free water to prevent arcing due to salts or reduced efficiency caused by EDTA or Tris containing buffers.

> "…run a titration experiment each time you begin transfecting a new nucleic acid, protein, or other molecule of interest."

Chapter 6
Optimizing Cell Density

Cell density can significantly affect transfection efficiency. Specifically, if the cells are too dilute or too dense at the time of harvest or at the time of transfection, then viability or efficiency may be compromised. This section provides information to help you achieve optimal cell densities—by cell type.

Cell Density Recommendations for Harvest

- Mammalian cells should be grown to a density of 70 to 80% confluency for adherent cells, or 1- to 2-million cells per ml for suspension cells, prior to harvesting for electroporation.
- Bacteria should be grown up to mid-log phase (around OD_{600}=0.5) prior to preparation of competent cells.

Cell Density Recommendations for Electroporation

- Mammalian cells 5- to 10-million cells per ml is an optimal cell density range for electroporation of most mammalian cells.
- Bacteria Self-prepared bacterial competent cells are typically in the density range of 1 X 10^{11} to 1 X 10^{12} cells per ml for electroporation.

Determining Optimal Cell Density

Consider running a titration experiment to determine the optimal number of cells to use.

To do this, simply test a range of cell densities, while keeping the transfectant amount, buffer type, and pulse generator settings constant. At the conclusion of the titration experiment, assess the transfection efficiency and cell viability for each cell density.

> “…[run] a titration experiment to determine the optimal number of cells to use.”

Chapter 7
Buffer Considerations

The buffer to suspend cells and transfectant at the time of transfection can markedly affect the viability of the cells or the stability of the transfectant. Here are some buffer recommendations based on cell type.

Recommended Buffers for Bacterial Cells

For Bacterial Cells, a low conductance, high resistance buffer is necessary to prevent arcing at the high voltages required to electroporate these cells.

Sterile, distilled water with 10% glycerol is commonly used for bacteria. The glycerol also serves as a cryoprotectant if you plan on freezing cells for future use.

Please see Chapter 10 for information about where to find our electrocompetent bacteria preparation protocol.

Recommended Buffers for Mammalian Cells

For Mammalian Cells, specialized, low conductance, electroporation buffers are recommended to minimize sample heating and to improve cell viability.

This type of buffer is especially helpful in high voltage applications or when scaling up to larger electroporation volumes. An example of a low conductance electroporation buffer is BTXpress Cytoporation Medium T.

For low voltage, cuvette-based applications, often times culture medium (for example RPMI, Opti-MEM) or buffered salt solutions (such as PBS or HBSS) are used. However, since culture media and buffered salts have high electrical conductance, they may cause arcing or undesirable heating.

Buffer Tips

When preparing your own electrocompetent cells, be sure to do enough cell washes to remove all residual salts from the growth medium.

It is critical to leave antibiotics out of electroporation media to avoid toxicity to cells.

> “Low conductance electroporation buffers minimize sample heating and improve cell viability.”

Chapter 8
Guidelines for Mammalian Cells

This section provides general guidelines for electroporation of mammalian cells, including best practice recommendations for generators, cuvettes, cell density/passage rate, and analysis timing.

Don't reinvent the wheel! If there is a protocol or published reference with electroporation parameters for your specific cell line/type (or for a similar cell type), start with it!

General Guidelines for Mammalian Cell Electroporation

Generator Selection

Most mammalian cells are transfected most efficiently with square wave generators. However, you can also electroporate them to less efficiency with an exponential decay wave generator. For certain mammalian cell types, such as stem cells, exponential decay wave generators are preferred.

Cuvette Gap Sizes

Generally 2 mm and 4 mm gap cuvettes are used for mammalian cells. However, 1 mm gap cuvettes may be used for smaller volumes, if the voltage is reduced accordingly, to achieve the desired field strength during electroporation.

- Typically field strengths of 400 to 1000 V/cm are used for mammalian cells, and a single pulse ranging from 5 to 25 ms is used for DNA electroporation.
- If using a 2 mm gap cuvette, the voltage ranges from 120 to 200 volts.
- If using a 4 mm gap cuvette, the voltage ranges from 170 to 300 volts.
- For small molecules, such as siRNA, high voltage microsecond pulse lengths may be needed.

Cell Density, Target Passage Number

Mammalian cells are best for transfection with a low passage number (ideally passage of 3 to 30) and actively growing. Using similar passage numbers between experiments can help to ensure reproducibility.

- High quality DNA that is low in endotoxin and concentrated to 1 to 5 mg/ml is ideal. Prepping DNA with a midi- to maxi-prep, using silica column or anion exchange column technology, is best. The DNA should be dissolved in nuclease-free water as Tris/EDTA solutions can reduce electroporation efficiency.

Results Analysis Timing

It also helps to determine the optimal time after electroporation to analyze your cells.

- For siRNA/miRNA knockdowns, typically 48 hours after transfection is the peak of mRNA knockdown. However, depending on the half-life of the protein, its peak knockdown may occur after that.

- Peak expression of protein from plasmid DNA or mRNA transcript usually falls within 12 to 72 hours after transfection. It is best to run a time-course experiment for each new construct to determine peak expression time.

Helpful References within this Document

- **Generator Selection**
  Refer to Chapter 1: Converting Between Square and Exponential Decay Wave

- **Cuvette Gap Sizes**
  Refer to Chapter 2: Converting Between Different Gap Sizes

- **Scaling**
  Refer to Chapter 3: Scaling Up and Down

- **Cell Density, Target Passage Sizes**
  Refer to Chapter 6: Optimizing Cell Density, for some discussion on optimal cell densities for growth prior to electroporation and suspension at the time of electroporation.

- **General Buffer Information**
  Refer to Chapter 7: Buffer Considerations, which includes information on using an electroporation buffer such as BTXpress Cytoporation Medium T to boost electroporation efficiency and cell viability.

> "…determine the optimal time after electroporation to analyze your cells—prior to electroporation".

Chapter 9
Guidelines for Bacterial Cells

This section provides best practice considerations for transforming bacteria including cell preparation, electroporation wave form considerations, cuvette selection, pulse length, and voltage.

Tips to boost your success transforming bacteria via electroporation

Electroporating cloning reactions, such as ligation mixtures, directly into bacteria can cause arcing and reduced transformation efficiencies.

- To reduce this risk, dilute ligations at least 1:5 with sterile nuclease-free water.

- Alternatively, you can remove salts by dialysis, ethanol precipitation, or spin column clean up.

Generator Selection

Bacteria typically are transformed most efficiently with exponential decay wave generators. However, you can also electroporate them with a square wave generator.

Cuvette Gap Sizes

Most often, 1 mm gap cuvettes are used for bacteria to achieve a high field strength during electroporation. 2 mm gap cuvettes or flatpack chambers may also be used for bacterial electroporation in larger volumes.

Target a field strength range of 15 to 25 kV/cm (or 1500 to 2500 V using 1 mm gap cuvettes).

Pulse Length

Typically a pulse length of around 5 ms works well for electroporation of bacteria. You may adjust the pulse length by increasing or decreasing the resistance and/or capacitance settings on your exponential decay wave generator.

- T=RC where the T is the Time Constant (time for the voltage to decay to 1/3 the set voltage), R is the Resistance and C is the Capacitance.

Notes: If you have purchased commercially available electrocompetent bacteria:

- Start with the manufacturer's recommended electroporation parameters, and then optimize from there.

If you are preparing your own electrocompetent bacteria:

- Grow your cells up to mid-log phase.
- Wash them well with a low conductance buffer.

The Additional Information and Resources section at the end of this Compendium provides information about our protocol for preparing electrocompetent bacteria and our protocol for optimizing bacterial transformation.

Helpful References within this Document

- **Generator Selection**
  Refer to Chapter 1: Converting Between Square and Exponential Decay Wave
- **Cuvette Gap Sizes**
  Refer to Chapter 2: Converting Between Different Gap Sizes
- **Scaling**
  Refer to Chapter 3: Scaling Up and Down
- **Cell Density, Target Passage Sizes**
  Refer to Chapter 6: Optimizing Cell Density
- **General Buffer Information**
  Refer to Chapter 7: Buffer Considerations

> "Bacteria typically are transformed most efficiently with exponential decay wave generators."

Additional Information & Resources

BTX Protocols

Electroporation Protocol Database

[https://www.btxonline.com/technical-resources/protocol-database.html]

Search for electroporation protocols by cell/tissue type, application/transfectant, citation and more.

Protocol for making Electrocompetent Bacteria

https://www.btxonline.com/media/wysiwyg/protocol_db/pdfs/MAKING%20ELECTROCOMPETENT%20BACTERIA.pdf

Protocol for Optimizing Bacterial Transformation

https://www.btxonline.com/media/wysiwyg/protocol_db/pdfs/PR0305a.pdf

Product Spotlight

BTX Gemini X2 Electroporator

BTX Gemini X2 Electroporator

[https://www.btxonline.com/products/electroporation-systems/gemini-twin-wave/gemini-twin-wave-electroporators.html?___SID=U]

Incorporating both square and exponential decay wave electroporation—in a single unit, the Gemini X2 is ideal for universal delivery of molecules of interest to any cell type in applications including in vitro cuvette, high throughput 96-well screening, CRISPR gene editing, in vivo tissue transfection and more.

Learn more about BTX Electroporation and Electrofusion Systems
[https://www.btxonline.com/catalogsearch/result/?cat=5&q=electroporation]

About the Author

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Michelle M. Ng is the Global Product Manager for the BTX brand of electroporation and electrofusion products and for the QuikPrep brand of sample preparation products at Harvard Bioscience. Prior to joining Harvard Bioscience, she worked as an Application Scientist at Thermo Fisher Scientific.

With more than 20 years of experience in molecular and cell biology, Michelle received her Ph.D. from the Department of Biology at Boston College. Through a fellowship from the American Cancer Society, Michelle conducted extensive postdoctoral research at the University of California, San Diego.

About BTX

BTX, a division of Harvard Bioscience, Inc., is a global leader in development and manufacture of innovative electroporation and electrofusion tools for the advancement of cell transfection and cell fusion. BTX remains at the forefront of this cutting-edge technology, offering a robust line of products, for which new applications are continually evolving. For more information please visit: www.btxonline.com.

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