

User's Manual

ECM[®] 630 Electroporation System



MA1 45-0051	ECM [®] 630 Electroporator only (110V)
MA1 45-0051int	ECM [®] 630 Electroporator only (220V)

BTX[®]

HARVARD APPARATUS

The Electroporation Experts

WEEE/RoHS Compliance Statement

EU Directives WEEE and RoHS

To Our Valued Customers:

We are committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. The European Union (EU) has enacted two Directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS). Over time, these Directives will be implemented in the national laws of each EU Member State.

Once the final national regulations have been put into place, recycling will be offered for our products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a “wheelie bin” symbol.

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive – Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments. Most of our products fall into either Category 8 or 9 and are currently exempt from the RoHS Directive. We will continue to monitor the application of the RoHS Directive to its products and will comply with any changes as they apply.



- **Do Not Dispose Product with Municipal Waste**
 - **Special Collection/Disposal Required**

Table of Contents

General Information:

Serial Number	2
Calibration	2
Warranty	2-3
Service	3-4
Repair Facilities and Parts	4

General Safety Summary.....5-6

Electrical & Technical Specifications7

General Specifications.....8

Introduction:

ECM® 630 Features	9
-------------------------	---

Operation: Getting Started10

Quick Start:

Installation	11
Connecting	11
Initializing.....	11
Instrument Controls.....	12-17

Operating Basics18-19

Advanced Operation: Programming20

Electroporation21

Applications:

Electroporation	22-23
-----------------------	-------

Appendix A: ECM® 630 Electrode

Operation Ranges.....	24-26
-----------------------	-------

Appendix B: ECM® 630 RC Time Constants.....27-38

Appendix C: Optimization Strategies.....39

Appendix D: Electrical Troubleshooting.....40

Appendix E: Experimental Troubleshooting41

Appendix F: Glossary of Electrical Terms42

Appendix G: Glossary of Biological &

Technical Terms	43-44
-----------------------	-------

Appendix H: Electroporation Generator

Compatibility	45
---------------------	----

Appendix I: Recommended Reading46

Appendix J: Accessories and Replacement Parts47

Appendix K: General Care and Cleaning.....48

Appendix L: Connecting Electrodes49-54

Appendix M: Plate Handler55-56

General Information

Serial Number

The serial number for the ECM® 630 is located on the rear of the instrument case. All inquiries concerning these products should refer to the serial numbers on the units.

Calibration

There is no calibration required for the ECM® 630.

Warranty

BTX - Harvard Apparatus warrants the ECM® 630 for a period of two years from the date of purchase. At its option, BTX - Harvard Apparatus will repair or replace the unit if it is found to be defective as to workmanship or materials. This warranty does not extend to any instrumentation which has been (a) subjected to misuse, neglect, accident or abuse, (b) repaired or altered by anyone other than BTX - HARVARD APPARATUS without BTX - HARVARD APPARATUS' express and prior approval, (c) used in violation of instructions furnished by BTX - HARVARD APPARATUS. This warranty extends only to the original customer purchaser.

IN NO EVENT SHALL BTX - HARVARD APPARATUS BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow exclusion or limitation of incidental or consequential damages so the above limitation or exclusion may not apply to you. **THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, OR FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE.** Some states do not allow this limitation on an implied warranty, so the above limitation may not apply to you.

Without limiting the generality of the foregoing, BTX - HARVARD APPARATUS shall not be liable for any claims of any kind whatsoever, as to the equipment delivered or for non-delivery of equipment, and whether or not based on negligence.

Warranty is void if the ECM® 630 is changed in any way from its original factory design or if repairs are attempted without written authorization by BTX - HARVARD APPARATUS.

Warranty is void if parts, connections or cell fusion chambers not manufactured by BTX - HARVARD APPARATUS are used with the ECM® 630.

General Information (Continued)

If a defect arises within the warranty period, promptly contact BTX – Harvard Apparatus, 84 October Hill Road, Building 7, Holliston, Massachusetts, USA 01746-1388 using our toll free number **1-800-272-2775** (US Only) or **508-893-8999**

(E-mail: techsupport.btx@harvardapparatus.com). Goods will not be accepted for return unless an RMA (Returned Materials Authorization) number has been issued by our customer service department. The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device.

This warranty gives you specific rights, and you may also have other rights, which vary from state to state.

Service

All service under the warranty will be made at the BTX - HARVARD APPARATUS, Holliston, Massachusetts facilities or an authorized service site. Owner will ship instrument prepaid to Holliston, Massachusetts, USA or the service site. BTX - HARVARD APPARATUS will return the instrument after servicing, freight prepaid to owner's address.

Obtaining Service:

Service During Warranty

1. Write or call the BTX - HARVARD APPARATUS Customer Support Group and describe the nature of the problem.
2. Carry out minor adjustments or tests as suggested by BTX - HARVARD APPARATUS.
3. If proper performance is not obtained, BTX - HARVARD APPARATUS will notify you to ship the instrument, prepaid, to its Service Department. The instrument will be repaired and returned at no charge for all customers in the continental United States.

Customers outside of the continental United States who have purchased our equipment from distributors should contact the distributor. If you have purchased your equipment from us, you should contact us directly. We will repair at no charge, but will not pay for shipment, documentation, etc. These charges will be billed at cost.

Note: Under no condition should the instrument or accessories be returned without prior approval from BTX - HARVARD APPARATUS. An RMA (Returned Materials Authorization) number must be obtained.

General Information (continued)

Out-Of-Warranty Service

Proceed exactly as for Warranty Service, above. If our Service Department can assist you by phone or correspondence, we will be glad to, at no charge.

Repair service will be billed on the basis of labor and materials. A complete statement of time spent and materials used will be supplied. Shipment to BTX - HARVARD APPARATUS should be prepaid. Your bill will include return shipment freight charges.

Disassembly by the user is prohibited. Service should only be carried out by experienced BTX - HARVARD APPARATUS technicians.

Repair Facilities and Parts

BTX - Harvard Apparatus stocks replacement and repair parts. When ordering, please describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample or drawing. We offer complete reconditioning service.

General Safety Summary

Review the following safety precautions to avoid injury and prevent damage to this product or any products connected to it. To avoid potential hazards, use this product only as specified.

Only qualified personnel should perform service procedures.

To Avoid Fire or Personal Injury

USE PROPER POWER CORD

Use only the power cord specified for this product and certified for the country of use.

CONNECT AND DISCONNECT PROPERLY

Do not connect or disconnect probes or test leads while they are connected to a power source.

GROUND THE PRODUCT

This product is grounded through the grounding conductor of the power cord. To avoid electric shock, the grounding conductor must be connected to earth ground. Before making connections to the output terminals of the product, ensure that the product is properly grounded.

OBSERVE ALL TERMINAL RATINGS

To avoid fire or shock hazard, observe all ratings and markings on the product. Consult the product manual for further ratings information before making connections to the product.

DO NOT OPERATE WITHOUT COVERS

Do not operate this product with covers or panels removed.

Use Proper Fuse. Use only the fuse type and rating specified for this product.

AVOID EXPOSURE TO CIRCUITRY

Do not touch exposed connections and components when power is present.

DO NOT OPERATE IN LOW IMPEDANCE

Sample: Load or Sample

If the electroporation samples have an impedance of less than 20 Ω the samples may arc and result in sample loss and potential damage to unit.

DO NOT OPERATE WITH SUSPECTED FAILURES

If you suspect there is damage to this product, have it inspected by qualified BTX service personnel.

PROVIDE PROPER VENTILATION

Refer to installation instructions for details on installing the product to ensure proper ventilation.

General Safety Summary (Continued)

DO NOT OPERATE IN WET/DAMP CONDITIONS

DO NOT OPERATE IN AN EXPLOSIVE ATMOSPHERE

KEEP PRODUCT SURFACES CLEAN AND DRY

Should you have any safety concerns, immediately contact BTX Technical Services (1-800-272-2775)

Safety Terms and Symbols:

Terms that appear in this manual:



WARNING. Warning statements identify conditions or practices that could result in injury or loss of life.



CAUTION. Caution statements identify conditions or practices that could result in damage to these products or other property.

Symbols that may appear on the products:



Danger
High
Voltage



Attention
Refer to
Manual



Protective
(Earth)
Terminal



Functional
Ground

Electrical & Technical Specifications

Standard Capabilities:

Display	Type: 20-character by 4-line liquid crystal Display. LED backlit
Power Source	
Voltage	100 to 240 Vac, 50 to 60 Hz, CAT II
Power Fusing	500 W (Pulsed), 50 W (Idle) 2.5 A, T rating 250 V

Environmental Characteristics:

Intended Use	Indoor use only
Operating Temperature	10° C to + 40° C
Cooling	Convection through metal case
Relative Humidity	60%
Altitude	2,000 m (operating)

Mechanical Characteristics:

Footprint	12.5" x 12.25" x 5.5" (W x D x H)
Weight	13.6 lbs (6.2 kg)
Controls	Single rotary encoder with integrated push button

General Specifications

Certifications and Compliances

Overvoltage Category:

CAT III: Products in this Category: Distribution-level mains, fixed installation.

CAT II: Local-level mains, applications, portable equipment.

CAT I: Signal levels in special equipment or parts of equipment, telecommunications, electronics.

Meets requirements of Directive 89/336/EEC for Electromagnetic Compatibility (EC) and Low-Voltage Directive 73/23/EEC for Product Safety.

Compliance was demonstrated to the following specifications as listed in the Official Journal of the European Communities:

EN 50081-1 Emissions

EN 55011 Class B Radiated and Conducted Emissions

EN 55082-1 Immunity

IEC 10004-2 Electrostatic Discharge Immunity

IEC 10004-3 RF Electromagnetic Field Immunity

IEC 10004-4 Electrical Fast Transient/Burst Immunity

Low Voltage Directive 73/23/EEC

<p>CAUTION FOR RESEARCH USE ONLY NOT FOR CLINICAL USE ON PATIENTS</p>

Introduction

The ECM® 630 is a new state of the art exponential decay wave electroporation system designed for in vitro and in vivo electroporation applications. This system incorporates features that make it the most technically advanced exponential decay electroporation system currently available.

ECM® 630 features include:

- The generator utilizes the new BTX Power Platform Technology design and novel digital user interface.
- The revolutionary Precision Pulse™ System provides the researcher unparalleled power in controlling the time constant.
- With the ability to deliver a maximum of 6000 A in the Low Voltage Mode, the ECM® 630 is the most powerful generator in its class.
- Voltage range of 10 V to 500 V with 1 V resolution and 1 μ F, 25 μ F to 3275 μ F in 25 μ F increments. 25 \square to 1575 \square , 25 \square resolution with “none” setting.
- Voltage range of 50 V to 2500 V with 5 V resolution and either 25 μ F or 50 μ F. 25 \square to 1575 \square with 25 \square resolution.
- Over 200 ECM® 600 protocols may be duplicated with this instrument.
- The additional ECM® 630 resistor selection “none” will allow researchers to reproduce protocols from competitive systems lacking resistor settings or reporting “unlimited” resistance.
- The ECM® 630 will perform the widest range of electroporation applications among commercially available electroporation generators.

Quick Start

Installation

1. Install on a bench or work table.
2. Allow a 1 to 2 inch clearance for proper cooling. It is normal for the instrument to be slightly warmer than its' operating environment.
3. Choose an outlet that is readily accessible.

Connecting

1. Insert female end of power cord into male power interface on the back panel of the ECM® 630.
2. Plug male end of power cord into appropriate electrical outlet.
3. Insert male banana plugs of the 630 B Safety Stand or alternative electrode device into High Voltage output located on front panel of the ECM® 630.

Initializing

1. Push the power switch located on the front panel of the ECM® 630.
2. The ECM® 630 will go through a series of self-test algorithms to test generator functionality.
3. The display will flash:



4. Following this initialization screen, the first time the instrument is initialized, the factory default display will then read:

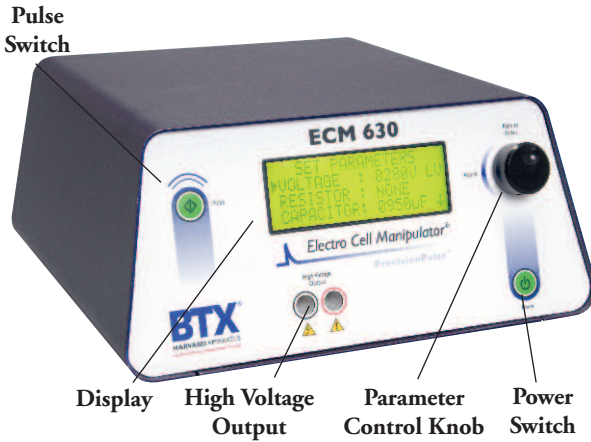


5. The first page of the Set Parameter Screen from the last time the ECM® 630 was used will be displayed each time the ECM® 630 is powered up after this initial start up.



Quick Start (Continued)

Instrument Controls



Power Switch

1. Electronic Power Switch located on the lower right front panel.
2. Press once to initialize the ECM® 630 and once more to turn off.



CAUTION: A stabilization period of 3 seconds is required after connecting to an outlet.

Pulse Switch

1. The electronic Pulse Switch on the upper left front panel is activated in the “ready” mode when the Set Parameters screen is displayed.
2. Once the start switch is activated, the generator will charge the capacitors to the preset voltage, then automatically deliver the pulse.
3. The maximum charge time is line voltage dependent and will typically be less than 10 seconds.
4. A pulse sequence may be aborted by pressing the Pulse switch a second time, before the charging is completed.
5. Following the delivery of a pulse, the Pulse switch can be pressed once to leave the feedback mode and return to the “ready” mode.

Quick Start (Continued)

Parameter Control Knob

The Parameter Control Knob is a rotary encoder controlling both the parameter under control and the value of the parameter under control.

1. The display will indicate which parameter is under control by the presence of an arrow to the left of the parameter.
2. To select a parameter to adjust, rotate the knob until the arrow is to the left of the desired parameter, then push to select. The arrow will move to the right of the value displayed for that parameter.
3. To adjust the value of a parameter under control, rotate the knob clockwise to increase the value and counter clockwise to decrease it. Once values are adjusted, push the knob to lock settings and arrow will return to left of parameters under control.
4. In order to move between screens, move the cursor to the bottom of the screen and rotate the knob clockwise to move to the next screen.
5. In order to move to the previous screen, move the cursor to the top of the screen and rotate the knob counterclockwise.

Display

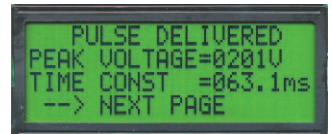
The ECM® 630 Display will show two possible screens, the Set Parameter Screen and the Pulse Delivered screen as well as various Status Messages.

Set Parameter Screen

1. The Set Parameters screen consists of two pages showing “Set Parameters” on the first line of each page.
2. The Voltage, Resistor (Resistance), and Capacitor (Capacitance) are shown on the first page and the Advanced Features including Save, View and Load are shown on the second page of the Set Parameters screen.
3. The ECM® 630 will beep when toggling from one page to another.

Voltage

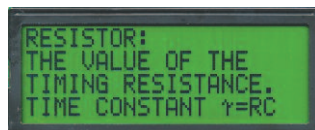
1. Voltage indicates the set voltage in volts.
2. The low voltage mode range is 10 V to 500 V in 1V increments and the high voltage mode range is 50 V to 2500 V in 5 V increments.
3. The mode is indicated by either LV for low voltage or HV for high voltage after the voltage value.
4. A beep is heard when transitioning between LV and HV modes..



Quick Start (Continued)

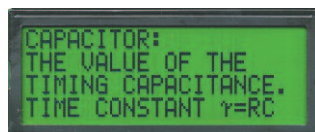
Resistor

1. Resistor indicates the set resistance in \square .
2. The resistance range is 25 \square to 1575 \square in 25 \square increments.
3. In the LV mode there is an additional resistor selection of “None”. This indicates that there is no timing resistor in the pulsing circuit, thus mimicking settings for competitive electroporators in which there is either no resistor to select, or the resistance is termed “unlimited.”



Capacitor

1. Capacitor indicates the set capacitance in microfarads (μF).
2. In the LV mode the capacitance range is 1 μF , 25 to 3275 μF in 25 μF increments.
3. In the HV mode the capacitance choice is 25 μF and 50 μF .
4. The capacitance and resistance control the time constant. Please refer to Appendix B for explanation and time constant charts.



Advanced Features

1. The second Set Parameters screen enables the user to save, view, and load up to three programs. Please note that current parameters will be overwritten once a program is loaded.
2. A default program is used to initialize the system. Program 1 is used to automatically store changes in parameters set each time the system is pulsed.
3. Programs #2 and #3 can be used to set and save experimental parameters for instantaneous use.

Save

1. To save the current set parameters as a program, push the parameter control knob to move the arrow to the left of the program number.
2. Rotate the knob to designate the appropriate program number, then push and hold the knob in, releasing after a confirmation screen is displayed.
3. The confirmation screen will read “Saved Current Parameters to Set N”(where N is a number from 1 to 3).
4. Push the parameter control knob again to return to the Set Parameters screen.

Quick Start (Continued)

View

1. To view the appropriate program parameters, push the parameter control knob to move the arrow to the left of the program number.
2. Select the appropriate program number by rotating the parameter control knob, then hold the knob in, releasing after a new screen is displayed.
3. The new screen will display the parameters currently stored under that program number.
4. Push the parameter control knob again to return to the Set Parameters screen.

Load

1. To load a saved program, push the parameter control knob to move the arrow to the left of the program number. Please note that in addition to three available programs, there is also the default program as outlined in the “Initializing” section.
2. Rotate the knob to change the program number.
3. Push and hold the knob in, releasing after a confirmation screen is displayed. The confirmation screen will read “Loaded Set N to Current Parameters” (where N is a number from 1 to 3).
4. Push the parameter control knob again to return to the Set Parameters screen.

On-Line Help

1. In the Set Parameter Mode, an On-Line Help function is available. On-Line Help provides a definition for all set parameters and advanced functions.
2. To use On-Line Help, rotate the parameter control knob so that the arrow is to the left of the parameter or feature of interest (not the value of the parameter).
3. Push the parameter control knob in and hold until the definition is displayed.
4. Push a second time to return to the Set Parameters screen.

Mode

Low voltage or high voltage range mode (display only, this function is controlled by the voltage parameter)

Voltage

Peak amplitude of the output pulse

Operation: Getting Started

Carefully open the box containing the ECM® 630 Electroporation System. Verify receipt of the following items:

ECM® 630 Pulse Generator (1)

Power Cord (1)

Model 630E Electronic Manual (1)

BTX Electroporation Systems may be customized with the addition of various electrodes and accessories. The following items complete a typical system order:

Model 630B Electroporation Safety Stand (1)

Model 660 Cuvette Rack (1)

Model 610 BTX Cuvettes Plus (10)

Model 620 BTX Cuvettes Plus (10)

Model 640 BTX Cuvettes Plus (10)

If you have ordered alternative or different items, please verify their receipt.

Quick Start (Continued)

Capacitor

The value of the timing capacitance. Time constant $t=RC$

Resistor

The value of the timing resistance. Time constant $t=RC$

Save

Save current setup parameters to nonvolatile memory

View

Show the setup parameters stored in nonvolatile memory

Load

Load setup parameters from memory to use

Status Messages

Following the initiation of a pulsing sequence, various status messages are displayed. The following status messages may be observed:

Charging, Pulsing, and Pulse Aborted During Charging.

Charging

As soon as the start switch is pressed, the “Charging” status message is displayed. The capacitors in the ECM® 630 are charged during the duration of this message.

Pulsing

1. Once the capacitors have reached the preset voltage level, they are discharged and the “Pulsing” status message is displayed for the duration of the pulse.
2. A click or a beep will be heard during the delivery of the pulse. The sound that is heard is a function of the pulse length. At pulse lengths less than 1 to 2 ms, clicks will likely be heard. At pulse lengths at or above 1 to 2 ms beeps should be heard.
3. Following the pulsing status message, the Pulse Delivered Screen is displayed.
4. Press the encoder or the Pulse switch to return to Set Parameters screen.

Pulsing Aborted During Charging

1. If the start switch is activated a second time prior to delivery of a pulse, the pulsing sequence is aborted and the “Pulsing Aborted” status message is displayed.
2. Press the encoder or the pulse switch once to get back to the Set Parameters screen.



Quick Start (Continued)

Pulse Delivered Screen

1. The Pulse Delivered Screen is displayed following the delivery of a pulse and is indicated by the display “Pulse Delivered” on the top line of the screen.
2. Press the knob to return to the Set Parameters Screen.
3. The Pulse Delivered screen displays the monitored peak Voltage and Time Constant on page 1 and the programmed Resistance and Capacitance on page 2. Please note that a pulse will be delivered and monitored, even if there is no output, so do not use this feature to verify that a pulse was delivered to your sample.
4. Always verify that your sample is connected to the HV output.

Voltage

1. Voltage indicates the peak voltage delivered. The voltage delivered is affected by the external load. (Sample)
2. Using heavy loads (low resistivity) will result in a slightly lower delivered voltage. This effect is normal and will be more pronounced with small capacitor settings.

Time Constant

Time Constant indicates the exponential decay time constant $1/e$ in ms, ms, or s.



Operating Basics

Use with Safety Stand 630B and BTX Disposable Electroporation Cuvettes Plus

1. Insert the safety stand banana plugs into the HV Output on the front panel of the ECM® 630.
2. Adjust the distance between the metal contacts using the black roller for your cuvette. (See: 630 B instruction sheet)
3. Press the green power switch to initialize the ECM® 630.
4. Rotate parameter control knob to move arrow to voltage and push to select. Rotate knob to adjust voltage. Push again to select that voltage.
5. Rotate parameter control knob to move arrow to Resistor and push to select. Rotate knob to adjust resistance. Push again to select that resistance.
6. Rotate parameter control knob to move arrow to Capacitor and push to select. Rotate knob to adjust the capacitance. Push again to select that capacitance.
7. Prepare sample, pipette into the appropriate BTX Disposable Cuvettes Plus, place the cuvette in the 630B Safety Stand and secure the safety cover.
8. Press the Start button. The ECM® 630 will charge and then deliver the electroporation pulse, while beeping.
9. Process sample. Do not forget to record appropriate parameters as displayed on the Pulse Delivered screens, for documentation purposes.
10. To return to “ready” mode, press the Pulse button.
11. To abort a pulse before delivery, press the Pulse button during the “charging” mode.

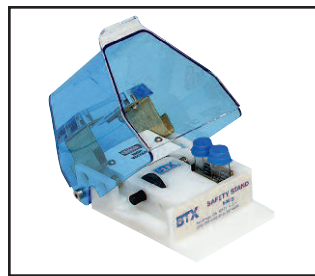
Use with Alternative Electrode Chambers and Applications

The ECM® 630 may be used to power all BTX electroporation cuvettes and electrodes. Follow the above instructions in conjunction with instructions provided for the specific electrode. Refer to Appendix A for graphical representation of operating ranges of various chambers and applicators with the ECM® 630.

Reproducing ECM® 600 Protocols

The ECM® 630 will reproduce all ECM® 600 protocols:

1. Set the voltage as outlined in the ECM® 600 protocol.
2. Calculate the RC time constant without factoring in the external load by multiplying the ECM® 600 protocol resistance and capacitance values and dividing by 1000 to give a result in msec.



630B
MA1 45-0207

Operating Basics (Continued)

3. Refer to Appendix B. Find the capacitance setting from step 2 and read down the column to find a RC time constant that closely matches. Extrapolate the new ECM® 630 resistance setting.
4. Set the capacitance from the ECM® 600 protocol and the new resistance extrapolated from Appendix B.
5. Follow experimental procedures while optimizing the voltage as outlined in Appendix C.

Reproducing Competitive System Protocols

The ECM® 630 is capable of reproducing most exponential competitive system protocols.

BioRad Gene Pulser® and Gene Pulser® II Protocols

For protocols using the Capacitance Extender, Capacitance Extender II, or the Capacitance Extender Plus, use the resistance setting “none”, reduce the voltage by 50 V and use the capacitance setting outlined in the protocol. For protocols using the Pulse Controller, Pulse Controller II, or Pulse Controller Plus, use a capacitance setting of 25 µF, reduce the voltage by 50 V and use the resistance settings outlined in the protocol. Optimization of voltage as outlined in Appendix C is recommended. For additional recommendations, please contact BTX Technical Support.

Other Competitive Exponential Decay Electroporation Protocols

Attempt to identify the resistance and capacitance as well as the voltage outlined in the protocol. Match these as closely to settings allowed with the ECM® 630. For low voltage protocols ($V \leq 500V$), if there is no resistance outlined, use the ECM® 630 resistance setting “none”. For additional recommendations, please contact BTX Technical Support.

Advanced Operation: Programming

1. Set parameters as outlined in Operating Basics.
2. Once presetting of parameters has been completed, rotate the parameter control knob until the save function has been reached.
3. Press knob in and release. Now rotate to select which program number the chosen settings (see "Preset Parameters" above) should be saved under. Program #1 is reserved for current parameters in active use and cannot be used for pre-set storage.
4. Push and hold knob in, releasing only after a new screen reading "Saved Current Parameters to Set N" is displayed. (N refers to program number designated in step 2)
5. Push the parameter control knob again to return to the Set Parameter Screen.
6. From the Set Parameter Screen, use the parameter control knob to rotate until the load function has been reached.
7. Push the knob in and release. Now rotate to select the appropriate program number.
8. Push and hold knob in, releasing only after a new screen reading "Loaded Set N to Current Parameters" is displayed.
9. Push the parameter control knob again to return to the Set Parameter Screen.

Electroporation

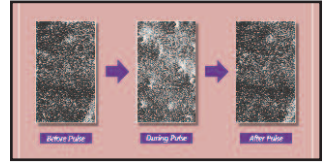
Electroporation is the application of controlled, pulsed electric fields to biological systems. If the biological system contains a lipid bilayer, such as is the case if the system is a suspension of cells or liposomes, the pulsed electric field may overcome the field potential of the lipid bilayer, resulting in a reversible breakdown of the bilayer and a resulting formation of temporal pores in the membrane. The pores formed are of the order of 40 to 120 nm. Most pores reseal within a few seconds, after allowing the transfer of materials into and out of the cells.

During a typical electroporation process, target cells and molecules are mixed together. When an electroporation pulse is delivered, the result is the formation of temporal pores. Before the pores reseal, the target molecules are observed to enter the cells. Upon resealing of the pores, the molecules become incorporated within the cell. The eventual target site depends on the application; for example, molecules can remain in the cytoplasm, interact with the membrane, and move into the nucleus.

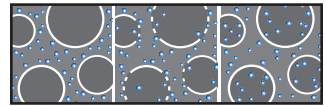
Applications for electroporation include permeabilization of virtually all cells to a wide variety of molecules and ions. The most common applications for electroporation are the transformation or transfection of cells with DNA or RNA. Other applications for electroporation include electroactivation, electroinsertion of proteins into cell membranes and electroextraction of molecules from cells. Although electroporation has mainly been used as a research tool, recent work has demonstrated its potential for clinical applications. Some areas being explored include:

- electrochemotherapy which involves electroporation for delivering chemotherapeutic agents directly to tumor cells
- encapsulation of drugs/genes into cells for their use as carrier systems
- transdermal delivery of drugs/genes
- gene therapy and delivery of drugs/genes with an electroporation catheter.

Electroporation can be characterized by waveform. BTX exponential decay waveform generators, such as the BTX ECM® 399 and ECM® 630 deliver an exponentially decaying pulse. The length of such a discharge waveform is commonly characterized by the time required for the initial voltage to decay to $1/e$ (roughly $1/3$) of the initial value. To achieve a desired pulse length, appropriate resistance and capacitance must be selected on the instrument. Voltage may be directly set on the instrument.



Pore Formation



Electroporation Process



Exponential Decay Wave

Applications

Electroporation

Bacteria and Yeast Electroporation

The most common application is transformation. Field strength and pulse length are critical parameters for reporting, optimization and troubleshooting bacterial and yeast applications.

Mammalian Cell Electroporation

Electroporation has been used successfully to introduce many different molecule types into cells. Most commonly, electroporation is used for the processes of transfection, in which nucleic acid (DNA and RNA), is introduced into cells.

Electroporation can be used to deliver oligonucleotides into cells for gene silencing or anti-sense applications. It can be used to deliver proteins into cells, even large enzymes such as restriction enzymes and antibodies, for various purposes. Peptides have also been electroincorporated. Smaller molecules have been incorporated into cells and liposomes, such as dyes, sugars and dNTP'S.

Electroporation has been used to study cellular activation processes, by electropermeabilizing cells to Ca^{2+} , Mg^{2+} and Na^+ . Electroporation is also used to electroinsert proteins into the cell membrane. Finally, electroporation has been used to introduce drugs, such as the chemotherapeutic agent bleomycin, into cancer cells, in vitro and in vivo.

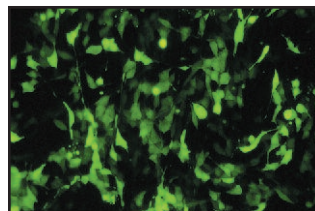
The use of low impedance buffers such as PBS may result in a voltage drop so that the actual peak voltage delivered to samples may be less than the set voltage.

With exponential decay generators, monitoring is necessary to identify the pulse length, or time constant, since this parameter may be very much dependent on the impedance of the sample (sample load).

When using complex and custom electroporation applicators and chambers, the electroporation waveform may be altered and monitoring is again strongly recommended.

Molecules Introduced by Electroporation

- DNA
- RNA
- dNTPS
- Enzymes
- Antibodies
- Other Proteins
- Peptides
- Dyes
- Sugars
- Ions
- Other Molecules



High GFP expression in Mouse PE501

Applications (Continued)

Plant Protoplast Electroporation

Electroporation has been used to introduce molecules into plant protoplasts, pollen and most recently, direct transfer into plant tissue (in vivo).

Other Electroporation Applications

1. Transgene incorporation, in which simple transfection of fish embryos has resulted in transgenic zebrafish.
2. Utilization of sperm as biological DNA carriers, in which pulsed fields cause the complexing of DNA to sperm, which then act as carriers upon fertilization.
3. Acrosome enhancement in which an exponential decay pulse enhances the acrosome reaction and facilitates fertilization.
4. Embryonic Stem Cell Chimeras, in which embryonic stem cell transfection, followed by micromanipulation into host blastomere, has resulted in chimeric mice.
5. Parthogenesis, in which a repetitive DC pulse stimulates an unfertilized egg to activate and divide as if fertilized, resulting in haploid and diploid embryos.

Appendix A: ECM® 630 Electrode Operation Ranges

Figures 1 - 6 display expected operating ranges for the ECM® 630 with various chambers and electrodes. The data lines in the following figures represent the arcing boundaries for the given electrode/chamber model, electroporation media, volume, and voltage/mode. The area at and above each line represents 100% arcing probability. The area beneath each line represents parameters that may lead to the delivery of a full pulse. This data was empirically determined in the BTX Application Laboratory.

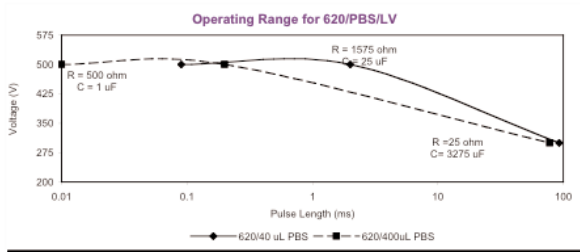


Figure 1
Operating Range for the ECM® 630 with Model 620 in LV with PBS at various volumes

2 mm gap cuvette
20 to 500 V
25 μ sec to 93 msec

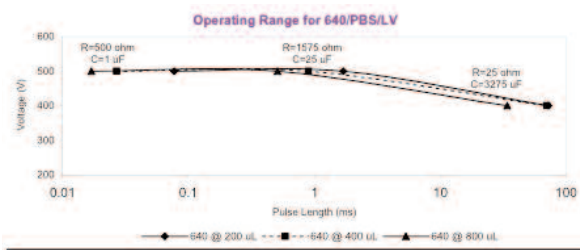


Figure 2
Operating Range for the ECM® 630 with Model 640 in LV with PBS at various volumes

4 mm gap cuvette
20 to 500 V
25 μ sec to 70 msec

Appendix A: ECM® 630 Electrode Operation Ranges (Continued)

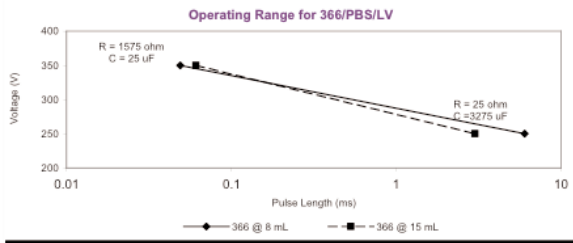


Figure 3
Operating Range for the ECM® 630 with 366 in LV with PBS at various volumes

Petri dish electrode
20 to 350 V
25 µsec to 6 msec

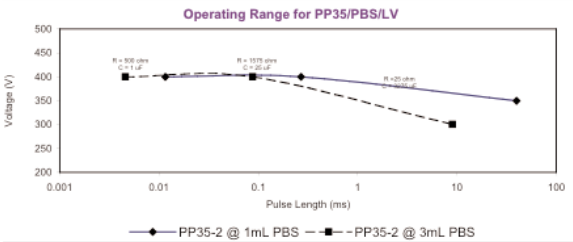


Figure 4
Operating Range for the ECM® 630 with PP35 in LV with PBS at various volumes

Petri Pulsar
20 to 400 V
10 µsec to 40 msec

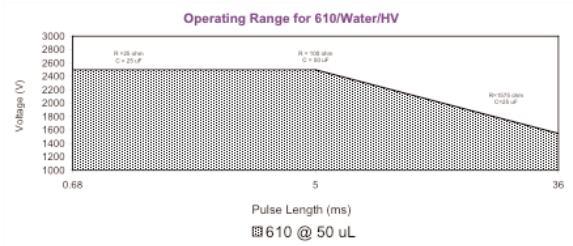


Figure 5
Operating Range for Model 610/H₂O/HV

1 mm gap cuvette
50 to 2500 V
680 µsec to 36 msec

Appendix A: ECM® 630 Electrode Operation Ranges (Continued)

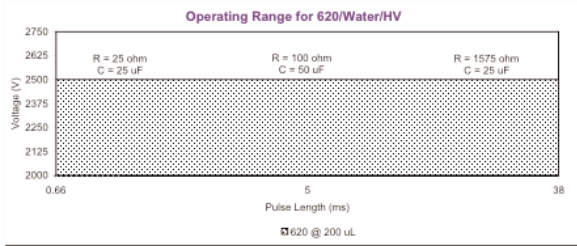


Figure 6
Operating Range for Model 620/H₂O/HV

2 mm gap cuvette
50 to 2500 V
660 µsec to 38 msec

Appendix B: ECM® 630 RC Time Constants

SEE PAGES 28 - 38

ECM® 630 Electroporation System

300	325	350	375	400	425	450	475	500	525	550	575	600
7.5	8.125	8.75	9.375	10	10.625	11.25	11.875	12.5	13.125	13.75	14.375	15
15	16.25	17.5	18.75	20	21.25	22.5	23.75	25	26.25	27.5	28.75	30
22.5	24.375	26.25	28.125	30	31.875	33.75	35.625	37.5	39.375	41.25	43.125	45
30	32.5	35	37.5	40	42.5	45	47.5	50	52.5	55	57.5	60
37.5	40.625	43.75	46.875	50	53.125	56.25	59.375	62.5	65.625	68.75	71.875	75
45	48.75	52.5	56.25	60	63.75	67.5	71.25	75	78.75	82.5	86.25	90
52.5	56.875	61.25	65.625	70	74.375	78.75	83.125	87.5	91.875	96.25	100.625	105
60	65	70	75	80	85	90	95	100	105	110	115	120
67.5	73.125	78.75	84.375	90	95.625	101.25	106.875	112.5	118.125	123.75	129.375	135
75	81.25	87.5	93.75	100	106.25	112.5	118.75	125	131.25	137.5	143.75	150
82.5	89.375	96.25	103.125	110	116.875	123.75	130.625	137.5	144.375	151.25	158.125	165
90	97.5	105	112.5	120	127.5	135	142.5	150	157.5	165	172.5	180
97.5	105.625	113.75	121.875	130	138.125	146.25	154.375	162.5	170.625	178.75	186.875	195
105	113.75	122.5	131.25	140	148.75	157.5	166.25	175	183.75	192.5	201.25	210
112.5	121.875	131.25	140.625	150	159.375	168.75	178.125	187.5	196.875	206.25	215.625	225
120	130	140	150	160	170	180	190	200	210	220	230	240
127.5	136.125	148.75	158.375	170	180.625	191.25	201.875	212.5	223.125	233.75	244.375	255
135	146.25	157.5	168.75	180	191.25	202.5	213.75	225	236.25	247.5	258.75	270
142.5	154.375	166.25	178.125	190	201.875	213.75	225.625	237.5	249.375	261.25	273.125	285
150	162.5	175	187.5	200	212.5	225	237.5	250	262.5	275	287.5	300
157.5	170.625	183.75	196.875	210	223.125	236.25	249.375	262.5	275.625	288.75	301.875	315
165	178.75	192.5	206.25	220	233.75	247.5	261.25	275	288.75	302.5	316.25	330
172.5	186.875	201.25	215.625	230	244.375	258.75	273.125	287.5	301.875	316.25	330.625	345
180	195	210	225	240	255	270	285	300	315	330	345	360
187.5	203.125	218.75	234.375	250	265.625	281.25	296.875	312.5	328.125	343.75	359.375	375
195	211.25	227.5	243.75	260	276.25	292.5	308.75	325	341.25	357.5	373.75	390
202.5	219.375	236.25	253.125	270	286.875	303.75	320.625	337.5	354.375	371.25	388.125	405
210	227.5	245	262.5	280	297.5	315	332.5	350	367.5	385	402.5	420
217.5	235.625	253.75	271.875	290	308.125	326.25	344.375	362.5	380.625	398.75	416.875	435
225	243.75	262.5	281.25	300	318.75	337.5	356.25	375	393.75	412.5	431.25	450
232.5	251.875	271.25	290.625	310	329.375	348.75	368.125	387.5	406.875	426.25	445.625	465
240	260	280	300	320	340	360	380	400	420	440	460	480
247.5	268.125	288.75	308.375	330	350.625	371.25	391.875	412.5	433.125	453.75	474.375	495
255	276.25	297.5	318.75	340	361.25	382.5	403.75	425	446.25	467.5	488.75	510
262.5	284.375	306.25	328.125	350	371.875	393.75	415.625	437.5	459.375	481.25	503.125	525
270	292.5	315	337.5	360	382.5	405	427.5	450	472.5	495	517.5	540
277.5	300.625	323.75	346.875	370	393.125	416.25	439.375	462.5	485.625	508.75	531.875	555
285	308.75	332.5	356.25	380	403.75	427.5	451.25	475	498.75	522.5	546.25	570
292.5	316.875	341.25	365.625	390	414.375	438.75	463.125	487.5	511.875	536.25	560.625	585
300	325	350	375	400	425	450	475	500	525	550	575	600
307.5	333.125	358.75	384.375	410	435.625	461.25	486.875	512.5	538.125	563.75	589.375	615
315	341.25	367.5	393.75	420	446.25	472.5	498.75	525	551.25	577.5	603.75	630
322.5	349.375	376.25	403.125	430	456.875	483.75	510.625	537.5	564.375	591.25	618.125	645
330	357.5	385	412.5	440	467.5	495	522.5	550	577.5	605	632.5	660
337.5	365.625	393.75	421.875	450	478.125	506.25	534.375	562.5	590.625	618.75	646.875	675
345	373.75	402.5	431.25	460	488.75	517.5	546.25	575	603.75	632.5	661.25	690
352.5	381.875	411.25	440.625	470	499.375	528.75	558.125	587.5	616.875	646.25	675.625	705
360	390	420	450	480	510	540	570	600	630	660	690	720
367.5	398.125	428.75	459.375	490	520.625	551.25	581.875	612.5	643.125	673.75	704.375	735
375	406.25	437.5	468.75	500	531.25	562.5	593.75	625	656.25	687.5	718.75	750
382.5	414.375	446.25	478.125	510	541.875	573.75	605.625	637.5	669.375	701.25	733.125	765
390	422.5	455	487.5	520	552.5	585	617.5	650	682.5	715	747.5	780
397.5	430.625	463.75	496.875	530	563.125	596.25	629.375	662.5	695.625	728.75	761.875	795
405	438.75	472.5	506.25	540	573.75	607.5	641.25	675	708.75	742.5	776.25	810
412.5	446.875	481.25	515.625	550	584.375	618.75	653.125	687.5	721.875	756.25	790.625	825
420	455	490	525	560	595	630	665	700	735	770	805	840
427.5	463.125	498.75	534.375	570	605.625	641.25	676.875	712.5	748.125	783.75	819.375	855
435	471.25	507.5	543.75	580	616.25	652.5	688.75	725	761.25	797.5	833.75	870
442.5	479.375	516.25	553.125	590	626.875	663.75	700.625	737.5	774.375	811.25	848.125	885
450	487.5	525	562.5	600	637.5	675	712.5	750	787.5	825	862.5	900
457.5	495.625	533.75	571.875	610	648.125	686.25	724.375	762.5	800.625	838.75	876.875	915
465	503.75	542.5	581.25	620	658.75	697.5	736.25	775	813.75	852.5	891.25	930
472.5	511.875	551.25	590.625	630	669.375	708.75	748.125	787.5	826.875	866.25	905.625	945

Appendix B: ECM® 630 RC Time Constants (Continued)

Low Voltage Mode RC

Resistor (ohm)	825	850	875	700	725	750	775	800	825	850	875	900
	15.625	16.25	16.875	17.5	18.125	18.75	19.375	20	20.625	21.25	21.875	22.5
	31.25	32.5	33.75	35	36.25	37.5	38.75	40	41.25	42.5	43.75	45
	46.875	48.75	50.625	52.5	54.375	56.25	58.125	60	61.875	63.75	65.625	67.5
	62.5	65	67.5	70	72.5	75	77.5	80	82.5	85	87.5	90
	78.125	81.25	84.375	87.5	90.625	93.75	96.875	100	103.125	106.25	109.375	112.5
	93.75	97.5	101.25	105	108.75	112.5	116.25	120	123.75	127.5	131.25	135
	109.375	113.75	118.125	122.5	126.875	131.25	135.625	140	144.375	148.75	153.125	157.5
	125	130	135	140	145	150	155	160	165	170	175	180
	140.625	146.25	151.875	157.5	163.125	168.75	174.375	180	186.625	191.25	196.875	202.5
	156.25	162.5	168.75	175	181.25	187.5	193.75	200	206.25	212.5	218.75	225
	171.875	178.75	185.625	192.5	199.375	206.25	213.125	220	226.875	233.75	240.625	247.5
	187.5	195	202.5	210	217.5	225	232.5	240	247.5	255	262.5	270
	203.125	211.25	219.375	227.5	235.625	243.75	251.875	260	258.125	276.25	284.375	292.5
	218.75	227.5	236.25	245	253.75	262.5	271.25	280	288.75	297.5	306.25	315
	234.375	243.75	253.125	262.5	271.875	281.25	290.625	300	308.375	318.75	328.125	337.5
	250	260	270	280	290	300	310	320	330	340	350	360
	265.625	276.25	286.875	297.5	308.125	318.75	329.375	340	350.625	361.25	371.875	382.5
	281.25	292.5	303.75	315	326.25	337.5	348.75	360	371.25	382.5	393.75	405
	296.875	308.75	320.625	332.5	344.375	356.25	368.125	380	391.875	403.75	415.625	427.5
	312.5	325	337.5	350	362.5	375	387.5	400	412.5	425	437.5	450
	328.125	341.25	354.375	367.5	380.625	393.75	406.875	420	433.125	446.25	459.375	472.5
	343.75	357.5	371.25	385	398.75	412.5	426.25	440	453.75	467.5	481.25	495
	359.375	373.75	388.125	402.5	416.875	431.25	445.625	460	474.375	488.75	503.125	517.5
	375	390	405	420	435	450	465	480	495	510	525	540
	390.625	406.25	421.875	437.5	453.125	468.75	484.375	500	515.625	531.25	546.875	562.5
	406.25	422.5	438.75	455	471.25	487.5	503.75	520	536.25	552.5	568.75	585
	421.875	438.75	455.625	472.5	489.375	506.25	523.125	540	556.875	573.75	590.625	607.5
	437.5	455	472.5	490	507.5	525	542.5	560	577.5	595	612.5	630
	453.125	471.25	489.375	507.5	525.625	543.75	561.875	580	598.125	616.25	634.375	652.5
	468.75	487.5	506.25	525	543.75	562.5	581.25	600	618.75	637.5	656.25	675
	484.375	503.75	523.125	542.5	561.875	581.25	600.625	620	638.375	658.75	678.125	697.5
	500	520	540	560	580	600	620	640	660	680	700	720
	515.625	536.25	556.875	577.5	598.125	618.75	639.375	660	680.625	701.25	721.875	742.5
	531.25	552.5	573.75	595	616.25	637.5	658.75	680	701.25	722.5	743.75	765
	546.875	568.75	590.625	612.5	634.375	656.25	678.125	700	721.875	743.75	765.625	787.5
	562.5	585	607.5	630	652.5	675	697.5	720	742.5	765	787.5	810
	578.125	601.25	624.375	647.5	670.625	693.75	716.875	740	763.125	786.25	809.375	832.5
	593.75	617.5	641.25	665	688.75	712.5	736.25	760	783.75	807.5	831.25	855
	609.375	633.75	658.125	682.5	706.875	731.25	755.625	780	804.375	828.75	853.125	877.5
	625	650	675	700	725	750	775	800	825	850	875	900
	640.625	666.25	691.875	717.5	743.125	768.75	794.375	820	845.625	871.25	896.875	922.5
	656.25	682.5	708.75	735	761.25	787.5	813.75	840	866.25	892.5	918.75	945
	671.875	698.75	725.625	752.5	779.375	806.25	833.125	860	886.875	913.75	940.625	967.5
	687.5	715	742.5	770	797.5	825	852.5	880	907.5	935	962.5	990
703.125	731.25	759.375	787.5	815.625	843.75	871.875	900	928.125	956.25	984.375	1012.5	
718.75	747.5	776.25	805	833.75	862.5	891.25	920	948.75	977.5	1006.25	1035	
734.375	763.75	793.125	822.5	851.875	881.25	910.625	940	969.375	998.75	1028.125	1057.5	
750	780	810	840	870	900	930	960	990	1020	1050	1080	
765.625	796.25	826.875	857.5	888.125	918.75	949.375	980	1010.625	1041.25	1071.875	1102.5	
781.25	812.5	843.75	875	906.25	937.5	968.75	1000	1031.25	1062.5	1093.75	1125	
796.875	828.75	860.625	892.5	924.375	956.25	988.125	1020	1051.875	1083.75	1115.625	1147.5	
812.5	845	877.5	910	942.5	975	1007.5	1040	1072.5	1105	1137.5	1170	
828.125	861.25	894.375	927.5	960.625	993.75	1026.875	1060	1093.125	1126.25	1159.375	1192.5	
843.75	877.5	911.25	945	978.75	1012.5	1046.25	1080	1113.75	1147.5	1181.25	1215	
859.375	893.75	928.125	962.5	996.875	1031.25	1065.625	1100	1134.375	1168.75	1203.125	1237.5	
875	910	945	980	1015	1050	1085	1120	1155	1190	1225	1260	
890.625	926.25	961.875	997.5	1033.125	1068.75	1104.375	1140	1175.625	1211.25	1246.875	1282.5	
906.25	942.5	978.75	1015	1051.25	1087.5	1123.75	1160	1196.25	1232.5	1268.75	1305	
921.875	958.75	995.625	1032.5	1069.375	1106.25	1143.125	1180	1216.875	1253.75	1290.625	1327.5	
937.5	975	1012.5	1050	1087.5	1125	1162.5	1200	1237.5	1275	1312.5	1350	
953.125	991.25	1029.375	1067.5	1105.625	1143.75	1181.875	1220	1258.125	1296.25	1334.375	1372.5	
968.75	1007.5	1046.25	1085	1123.75	1162.5	1201.25	1240	1278.75	1317.5	1356.25	1395	
984.375	1023.75	1063.125	1102.5	1141.875	1181.25	1220.625	1260	1299.375	1338.75	1378.125	1417.5	

ECM® 630 Electroporation System

Capacitor (uF)												
1600	1625	1650	1675	1700	1725	1750	1775	1800	1825	1850	1875	1900
40	40.625	41.25	41.875	42.5	43.125	43.75	44.375	45	45.625	46.25	46.875	47.5
60	81.25	82.5	83.75	85	86.25	87.5	88.75	90	91.25	92.5	93.75	95
120	121.875	123.75	125.625	127.5	129.375	131.25	133.125	135	136.875	138.75	140.625	142.5
160	162.5	165	167.5	170	172.5	175	177.5	180	182.5	185	187.5	190
200	203.125	206.25	209.375	212.5	215.625	218.75	221.875	225	228.125	231.25	234.375	237.5
240	243.75	247.5	251.25	255	258.75	262.5	266.25	270	273.75	277.5	281.25	285
280	284.375	288.75	293.125	297.5	301.875	306.25	310.625	315	319.375	323.75	328.125	332.5
320	325	330	335	340	345	350	355	360	365	370	375	380
360	365.625	371.25	376.875	382.5	388.125	393.75	399.375	405	410.625	416.25	421.875	427.5
400	405.25	412.5	418.75	425	431.25	437.5	443.75	450	456.25	462.5	468.75	475
440	446.875	453.75	460.625	467.5	474.375	481.25	488.125	495	501.875	508.75	515.625	522.5
480	487.5	495	502.5	510	517.5	525	532.5	540	547.5	555	562.5	570
520	528.125	536.25	544.375	552.5	560.625	568.75	576.875	585	593.125	601.25	609.375	617.5
560	568.75	577.5	586.25	595	603.75	612.5	621.25	630	638.75	647.5	656.25	665
600	608.375	618.75	628.125	637.5	646.875	656.25	665.625	675	684.375	693.75	703.125	712.5
640	650	660	670	680	690	700	710	720	730	740	750	760
680	690.625	701.25	711.875	722.5	733.125	743.75	754.375	765	775.625	786.25	796.875	807.5
720	731.25	742.5	753.75	765	776.25	787.5	798.75	810	821.25	832.5	843.75	855
760	771.875	783.75	795.625	807.5	819.375	831.25	843.125	855	866.875	878.75	890.625	902.5
800	812.5	825	837.5	850	862.5	875	887.5	900	912.5	925	937.5	950
840	853.125	866.25	879.375	892.5	905.625	918.75	931.875	945	958.125	971.25	984.375	997.5
880	893.75	907.5	921.25	935	948.75	962.5	976.25	990	1003.75	1017.5	1031.25	1045
920	934.375	948.75	963.125	977.5	991.875	1006.25	1020.625	1035	1049.375	1063.75	1078.125	1092.5
960	975	990	1005	1020	1035	1050	1065	1080	1095	1110	1125	1140
1000	1015.625	1031.25	1046.875	1062.5	1078.125	1093.75	1109.375	1125	1140.625	1156.25	1171.875	1187.5
1040	1058.25	1072.5	1086.75	1105	1121.25	1137.5	1153.75	1170	1186.25	1202.5	1218.75	1235
1080	1096.875	1113.75	1130.625	1147.5	1164.375	1181.25	1198.125	1215	1231.875	1248.75	1265.625	1282.5
1120	1137.5	1155	1172.5	1190	1207.5	1225	1242.5	1260	1277.5	1295	1312.5	1330
1160	1178.125	1196.25	1214.375	1232.5	1250.625	1268.75	1286.875	1305	1323.125	1341.25	1359.375	1377.5
1200	1218.75	1237.5	1256.25	1275	1293.75	1312.5	1331.25	1350	1368.75	1387.5	1406.25	1425
1240	1259.375	1278.75	1298.125	1317.5	1336.875	1356.25	1375.625	1395	1414.375	1433.75	1453.125	1472.5
1280	1300	1320	1340	1360	1380	1400	1420	1440	1460	1480	1500	1520
1320	1340.625	1361.25	1381.875	1402.5	1423.125	1443.75	1464.375	1485	1505.625	1526.25	1546.875	1567.5
1360	1381.25	1402.5	1423.75	1445	1466.25	1487.5	1508.75	1530	1551.25	1572.5	1593.75	1615
1400	1421.875	1443.75	1465.625	1487.5	1508.375	1531.25	1553.125	1575	1596.875	1618.75	1640.625	1662.5
1440	1462.5	1485	1507.5	1530	1552.5	1575	1597.5	1620	1642.5	1665	1687.5	1710
1480	1503.125	1526.25	1549.375	1572.5	1595.625	1618.75	1641.875	1665	1688.125	1711.25	1734.375	1757.5
1520	1543.75	1567.5	1591.25	1615	1638.75	1662.5	1686.25	1710	1733.75	1757.5	1781.25	1805
1560	1584.375	1608.75	1633.125	1657.5	1681.875	1706.25	1730.625	1755	1779.375	1803.75	1828.125	1852.5
1600	1625	1650	1675	1700	1725	1750	1775	1800	1825	1850	1875	1900
1640	1665.625	1691.25	1716.875	1742.5	1768.125	1793.75	1819.375	1845	1870.625	1896.25	1921.875	1947.5
1680	1706.25	1732.5	1758.75	1785	1811.25	1837.5	1863.75	1890	1916.25	1942.5	1968.75	1995
1720	1746.875	1773.75	1800.625	1827.5	1854.375	1881.25	1908.125	1935	1961.875	1988.75	2015.625	2042.5
1760	1787.5	1815	1842.5	1870	1897.5	1925	1952.5	1980	2007.5	2035	2062.5	2090
1800	1828.125	1856.25	1884.375	1912.5	1940.625	1968.75	1996.875	2025	2053.125	2081.25	2109.375	2137.5
1840	1868.75	1897.5	1926.25	1955	1983.75	2012.5	2041.25	2070	2098.75	2127.5	2156.25	2185
1880	1909.375	1938.75	1968.125	1997.5	2026.875	2056.25	2085.625	2115	2144.375	2173.75	2203.125	2232.5
1920	1950	1980	2010	2040	2070	2100	2130	2160	2190	2220	2250	2280
1960	1990.625	2021.25	2051.875	2082.5	2113.125	2143.75	2174.375	2205	2235.625	2266.25	2296.875	2327.5
2000	2031.25	2062.5	2093.75	2125	2156.25	2187.5	2218.75	2250	2281.25	2312.5	2343.75	2375
2040	2071.875	2103.75	2135.625	2167.5	2199.375	2231.25	2263.125	2295	2326.875	2358.75	2390.625	2422.5
2080	2112.5	2145	2177.5	2210	2242.5	2275	2307.5	2340	2372.5	2405	2437.5	2470
2120	2153.125	2186.25	2219.375	2252.5	2285.625	2318.75	2351.875	2385	2418.125	2451.25	2484.375	2517.5
2160	2193.75	2227.5	2261.25	2295	2328.75	2362.5	2396.25	2430	2463.75	2497.5	2531.25	2565
2200	2234.375	2268.75	2303.125	2337.5	2371.875	2406.25	2440.625	2475	2509.375	2543.75	2578.125	2612.5
2240	2275	2310	2345	2380	2415	2450	2485	2520	2555	2590	2625	2660
2280	2315.625	2351.25	2386.875	2422.5	2458.125	2493.75	2529.375	2565	2600.625	2636.25	2671.875	2707.5
2320	2356.25	2392.5	2428.75	2465	2501.25	2537.5	2573.75	2610	2646.25	2682.5	2718.75	2755
2360	2396.875	2433.75	2470.625	2507.5	2544.375	2581.25	2618.125	2655	2691.875	2728.75	2765.625	2802.5
2400	2437.5	2475	2512.5	2550	2587.5	2625	2662.5	2700	2737.5	2775	2812.5	2850
2440	2478.125	2516.25	2554.375	2592.5	2630.625	2668.75	2706.875	2745	2783.125	2821.25	2859.375	2897.5
2480	2518.75	2557.5	2596.25	2635	2673.75	2712.5	2751.25	2790	2828.75	2867.5	2906.25	2945
2520	2559.375	2598.75	2638.125	2677.5	2716.875	2756.25	2795.625	2835	2874.375	2913.75	2953.125	2992.5

ECM® 630 Electroporation System

2250	2275	2300	2325	2350	2375	2400	2425	2450	2475	2500	2525	2550
56.25	56.875	57.5	58.125	58.75	59.375	60	60.625	61.25	61.875	62.5	63.125	63.75
112.5	113.75	115	116.25	117.5	118.75	120	121.25	122.5	123.75	125	126.25	127.5
168.75	170.625	172.5	174.375	176.25	178.125	180	181.875	183.75	185.625	187.5	189.375	191.25
225	227.5	230	232.5	235	237.5	240	242.5	245	247.5	250	252.5	255
281.25	284.375	287.5	290.625	293.75	296.875	300	303.125	306.25	309.375	312.5	315.625	318.75
337.5	341.25	345	348.75	352.5	356.25	360	363.75	367.5	371.25	375	378.75	382.5
393.75	398.125	402.5	406.875	411.25	415.625	420	424.375	428.75	433.125	437.5	441.875	446.25
450	455	460	465	470	475	480	485	490	495	500	505	510
506.25	511.875	517.5	523.125	528.75	534.375	540	545.625	551.25	556.875	562.5	568.125	573.75
562.5	568.75	575	581.25	587.5	593.75	600	606.25	612.5	618.75	625	631.25	637.5
618.75	625.625	632.5	639.375	646.25	653.125	660	666.875	673.75	680.625	687.5	694.375	701.25
675	682.5	690	697.5	705	712.5	720	727.5	735	742.5	750	757.5	765
731.25	738.375	747.5	755.625	763.75	771.875	780	788.125	796.25	804.375	812.5	820.625	828.75
787.5	795.25	805	813.75	822.5	831.25	840	848.75	857.5	866.25	875	883.75	892.5
843.75	853.125	862.5	871.875	881.25	890.625	900	909.375	918.75	928.125	937.5	946.875	956.25
900	910	920	930	940	950	960	970	980	990	1000	1010	1020
956.25	966.875	977.5	988.125	998.75	1009.375	1020	1030.625	1041.25	1051.875	1062.5	1073.125	1083.75
1012.5	1023.75	1035	1046.25	1057.5	1068.75	1080	1091.25	1102.5	1113.75	1125	1136.25	1147.5
1068.75	1080.625	1092.5	1104.375	1116.25	1128.125	1140	1151.875	1163.75	1175.625	1187.5	1199.375	1211.25
1125	1137.5	1150	1162.5	1175	1187.5	1200	1212.5	1225	1237.5	1250	1262.5	1275
1181.25	1194.375	1207.5	1220.625	1233.75	1246.875	1260	1273.125	1286.25	1299.375	1312.5	1325.625	1338.75
1237.5	1251.25	1265	1278.75	1292.5	1306.25	1320	1333.75	1347.5	1361.25	1375	1388.75	1402.5
1293.75	1308.125	1322.5	1336.875	1351.25	1365.625	1380	1394.375	1408.75	1423.125	1437.5	1451.875	1466.25
1350	1365	1380	1395	1410	1425	1440	1455	1470	1485	1500	1515	1530
1406.25	1421.875	1437.5	1453.125	1468.75	1484.375	1500	1515.625	1531.25	1546.875	1562.5	1578.125	1593.75
1462.5	1478.75	1495	1511.25	1527.5	1543.75	1560	1576.25	1592.5	1608.75	1625	1641.25	1657.5
1518.75	1535.625	1552.5	1569.375	1586.25	1603.125	1620	1636.875	1653.75	1670.625	1687.5	1704.375	1721.25
1575	1592.5	1610	1627.5	1645	1662.5	1680	1697.5	1715	1732.5	1750	1767.5	1785
1631.25	1649.375	1667.5	1685.625	1703.75	1721.875	1740	1758.125	1776.25	1794.375	1812.5	1830.625	1848.75
1687.5	1706.25	1725	1743.75	1762.5	1781.25	1800	1818.75	1837.5	1856.25	1875	1893.75	1912.5
1743.75	1763.125	1782.5	1801.875	1821.25	1840.625	1860	1879.375	1898.75	1918.125	1937.5	1956.875	1976.25
1800	1820	1840	1860	1880	1900	1920	1940	1960	1980	2000	2020	2040
1856.25	1876.875	1897.5	1918.125	1938.75	1959.375	1980	2000.625	2021.25	2041.875	2062.5	2083.125	2103.75
1912.5	1933.75	1955	1976.25	1997.5	2018.75	2040	2061.25	2082.5	2103.75	2125	2146.25	2167.5
1968.75	1990.625	2012.5	2034.375	2056.25	2078.125	2100	2121.875	2143.75	2165.625	2187.5	2209.375	2231.25
2025	2047.5	2070	2092.5	2115	2137.5	2160	2182.5	2205	2227.5	2250	2272.5	2295
2081.25	2104.375	2127.5	2150.625	2173.75	2196.875	2220	2243.125	2266.25	2289.375	2312.5	2335.625	2358.75
2137.5	2161.25	2185	2208.75	2232.5	2256.25	2280	2303.75	2327.5	2351.25	2375	2398.75	2422.5
2193.75	2218.125	2242.5	2266.875	2291.25	2315.625	2340	2364.375	2388.75	2413.125	2437.5	2461.875	2486.25
2250	2275	2300	2325	2350	2375	2400	2425	2450	2475	2500	2525	2550
2306.25	2331.875	2357.5	2383.125	2408.75	2434.375	2460	2485.625	2511.25	2536.875	2562.5	2588.125	2613.75
2362.5	2388.75	2415	2441.25	2467.5	2493.75	2520	2546.25	2572.5	2598.75	2625	2651.25	2677.5
2418.75	2445.625	2472.5	2499.375	2526.25	2553.125	2580	2606.875	2633.75	2660.625	2687.5	2714.375	2741.25
2475	2502.5	2530	2557.5	2585	2612.5	2640	2667.5	2695	2722.5	2750	2777.5	2805
2531.25	2559.375	2587.5	2615.625	2643.75	2671.875	2700	2728.125	2756.25	2784.375	2812.5	2840.625	2868.75
2587.5	2616.25	2645	2673.75	2702.5	2731.25	2760	2788.75	2817.5	2846.25	2875	2903.75	2932.5
2643.75	2673.125	2702.5	2731.875	2761.25	2790.625	2820	2849.375	2878.75	2908.125	2937.5	2966.875	2996.25
2700	2730	2760	2790	2820	2850	2880	2910	2940	2970	3000	3030	3060
2756.25	2786.875	2817.5	2848.125	2878.75	2909.375	2940	2970.625	3001.25	3031.875	3062.5	3093.125	3123.75
2812.5	2843.75	2875	2906.25	2937.5	2968.75	3000	3031.25	3062.5	3093.75	3125	3156.25	3187.5
2868.75	2900.625	2932.5	2964.375	2996.25	3028.125	3060	3091.875	3123.75	3155.625	3187.5	3219.375	3251.25
2925	2957.5	2990	3022.5	3055	3087.5	3120	3152.5	3185	3217.5	3250	3282.5	3315
2981.25	3014.375	3047.5	3080.625	3113.75	3146.875	3180	3213.125	3246.25	3279.375	3312.5	3345.625	3378.75
3037.5	3071.25	3105	3138.75	3172.5	3206.25	3240	3273.75	3307.5	3341.25	3375	3408.75	3442.5
3093.75	3128.125	3162.5	3196.875	3231.25	3265.625	3300	3334.375	3368.75	3403.125	3437.5	3471.875	3506.25
3150	3185	3220	3255	3290	3325	3360	3395	3430	3465	3500	3535	3570
3206.25	3241.875	3277.5	3313.125	3348.75	3384.375	3420	3455.625	3491.25	3526.875	3562.5	3598.125	3633.75
3262.5	3298.75	3335	3371.25	3407.5	3443.75	3480	3516.25	3552.5	3588.75	3625	3661.25	3697.5
3318.75	3355.625	3392.5	3429.375	3466.25	3503.125	3540	3576.875	3613.75	3650.625	3687.5	3724.375	3761.25
3375	3412.5	3450	3487.5	3525	3562.5	3600	3637.5	3675	3712.5	3750	3787.5	3825
3431.25	3469.375	3507.5	3545.625	3583.75	3621.875	3660	3698.125	3736.25	3774.375	3812.5	3850.625	3888.75
3487.5	3526.25	3565	3603.75	3642.5	3681.25	3720	3758.75	3797.5	3836.25	3875	3913.75	3952.5
3543.75	3583.125	3622.5	3661.875	3701.25	3740.625	3780	3819.375	3858.75	3898.125	3937.5	3976.875	4016.25

Appendix B: ECM® 630 RC Time Constants (Continued)

**Low Voltage
Mode RC**

	3225	3250	3275
	80.625	81.25	81.875
	161.25	162.5	163.75
	241.875	243.75	245.625
	322.5	325	327.5
	403.125	406.25	409.375
	483.75	487.5	491.25
	564.375	568.75	573.125
	645	650	655
	725.625	731.25	736.875
	806.25	812.5	818.75
	886.875	893.75	900.625
	967.5	975	982.5
	1048.125	1056.25	1064.375
	1128.75	1137.5	1148.25
	1209.375	1218.75	1228.125
	1290	1300	1310
	1370.625	1381.25	1391.875
	1451.25	1462.5	1473.75
	1531.875	1543.75	1555.625
	1612.5	1625	1637.5
	1693.125	1706.25	1719.375
	1773.75	1787.5	1801.25
	1854.375	1868.75	1883.125
	1935	1950	1965
	2015.625	2031.25	2046.875
	2096.25	2112.5	2128.75
	2176.875	2193.75	2210.625
	2257.5	2275	2292.5
	2338.125	2356.25	2374.375
	2418.75	2437.5	2456.25
	2499.375	2518.75	2538.125
	2580	2600	2620
	2660.625	2681.25	2701.875
	2741.25	2762.5	2783.75
	2821.875	2843.75	2865.625
	2902.5	2925	2947.5
	2983.125	3006.25	3029.375
	3063.75	3087.5	3111.25
	3144.375	3168.75	3193.125
	3225	3250	3275
	3305.625	3331.25	3356.875
	3386.25	3412.5	3438.75
	3466.875	3493.75	3520.625
	3547.5	3575	3602.5
	3628.125	3656.25	3684.375
	3708.75	3737.5	3766.25
	3789.375	3818.75	3848.125
	3870	3900	3930
	3950.625	3981.25	4011.875
	4031.25	4062.5	4093.75
	4111.875	4143.75	4175.625
	4192.5	4225	4257.5
	4273.125	4306.25	4339.375
	4353.75	4387.5	4421.25
	4434.375	4468.75	4503.125
	4515	4550	4565
	4595.625	4631.25	4666.875
	4676.25	4712.5	4748.75
	4756.875	4793.75	4830.625
	4837.5	4875	4912.5
	4918.125	4956.25	4994.375
	4998.75	5037.5	5076.25
	5079.375	5118.75	5158.125

Capacitor (uF)

25.00	0.63	1.25	50.00
50.00	1.25	2.50	75.00
75.00	1.88	3.75	100.00
100.00	2.50	5.00	125.00
125.00	3.13	6.25	150.00
150.00	3.75	7.50	175.00
175.00	4.38	8.75	200.00
200.00	5.00	10.00	225.00
225.00	5.63	11.25	250.00
250.00	6.25	12.50	275.00
275.00	6.88	13.75	300.00
300.00	7.50	15.00	325.00
325.00	8.13	16.25	350.00
350.00	8.75	17.50	375.00
375.00	9.38	18.75	400.00
400.00	10.00	20.00	425.00
425.00	10.63	21.25	450.00
450.00	11.25	22.50	475.00
475.00	11.88	23.75	500.00
500.00	12.50	25.00	525.00
525.00	13.13	26.25	550.00
550.00	13.75	27.50	575.00
575.00	14.38	28.75	600.00
600.00	15.00	30.00	625.00
625.00	15.63	31.25	650.00
650.00	16.25	32.50	675.00
675.00	16.88	33.75	700.00
700.00	17.50	35.00	725.00
725.00	18.13	36.25	750.00
750.00	18.75	37.50	775.00
775.00	19.38	38.75	800.00
800.00	20.00	40.00	825.00
825.00	20.63	41.25	850.00
850.00	21.25	42.50	875.00
875.00	21.88	43.75	900.00
900.00	22.50	45.00	925.00
925.00	23.13	46.25	950.00
950.00	23.75	47.50	975.00
975.00	24.38	48.75	1000.00
1000.00	25.00	50.00	1025.00
1025.00	25.63	51.25	1050.00
1050.00	26.25	52.50	1075.00
1075.00	26.88	53.75	1100.00
1100.00	27.50	55.00	1125.00
1125.00	28.13	56.25	1150.00
1150.00	28.75	57.50	1175.00
1175.00	29.38	58.75	1200.00
1200.00	30.00	60.00	1225.00
1225.00	30.63	61.25	1250.00
1250.00	31.25	62.50	1275.00
1275.00	31.88	63.75	1300.00
1300.00	32.50	65.00	1325.00
1325.00	33.13	66.25	1350.00
1350.00	33.75	67.50	1375.00
1375.00	34.38	68.75	1400.00
1400.00	35.00	70.00	1425.00
1425.00	35.63	71.25	1450.00
1450.00	36.25	72.50	1475.00
1475.00	36.88	73.75	1500.00
1500.00	37.50	75.00	1525.00
1525.00	38.13	76.25	1550.00
1550.00	38.75	77.50	1575.00
1575.00	39.38	78.75	

Resistor (ohm)

Resistor (ohm)

Appendix C: Optimization Strategies

General

The success of electro cell manipulation (ECM) lies in selecting appropriate ECM systems capable of delivering the pulses suitable for the cell being electromanipulated. One, or several pulses of the appropriate field strength, pulse length, and wave shape may be required for this purpose.

The key to success with electroporation-based technologies involves a proper combination of biological, physical, chemical, and pulse parameters. In general, cells must be in mid-logarithmic growth for optimal electroporation. Various temperature regimens have been described. It has been shown that a variety of chemical techniques may increase electroporation efficiencies, including addition of EDTA, DMSO, intracellular salts, and serum before or after the pulse. Optimizing protocols abound. Analysis of these optimization regimens has led to proposals of universal protocols, involving very limited optimization over a narrow range.

Electroporation

1. Vary the voltage in order to vary the field strength, keeping other parameters constant. Assay sample for both viability and endpoint. Plot the field strength versus both viability and endpoint and extrapolate the optimal field strength (voltage divided by gap size) and voltage.
2. Vary the capacitance/resistance/sample volume at the optimal voltage setting in order to vary the pulse length (time constant) for exponential decay instruments. Directly vary square wave instrument pulse length. Assay sample for both viability and endpoint. Plot the pulse length versus both viability and endpoint and extrapolate the optimal pulse length/parameters.
3. For multiple pulsing systems/protocols, vary the number of pulses at the optimal field strength and pulse length. Assay sample for both viability and endpoint. Plot the number of pulses versus both viability and endpoint, and extrapolate the optimal number of pulses.

Appendix D: Electrical Troubleshooting

Instrument Does Not Power Up

Verify that the power cord is fully inserted in the instrument and in the wall outlet. Verify that the fuse is not blown. Disconnect power cord from the instrument before removing the fuse holder. Replace the fuse, if necessary, with same rated fuse as indicated on back panel.

Unanticipated RC time constants or peak voltage output

Un-anticipated RC time constant or peak output voltage may be a sign that appropriate capacitors are not being selected or are not fully charging. Please remember that the external load (sample) reduces the expected time constant and voltage to various degrees. If you believe there is a problem, contact BTX Technical Support for immediate consultation.

LCD Error Messages

The ECM® 630 is constantly monitoring the parameters of some of its internal circuitry. In the case of a malfunction, one of the following messages will appear on the display. Note the instructions on the following page used to confirm the absence of a pulse. In this case, call BTX Technical Support.

EEPROM Failure

The unit has detected a malfunction in its internal memory system. The validity of the data might be compromised. Turning or pressing the knob will bring the Set Parameters screen. Verify carefully every setpoint before pulsing. This verification is performed during power up and every time that data is loaded from memory. Contact BTX Technical Support if this error message is displayed again, after a power up sequence.

Pulsing Aborted Charge Failure

The unit did not charge its selected capacitor bank. Turn or press the encoder knob. Disconnect the load from the HV connector. Select a different capacitor and press the pulse button. Contact BTX® Technical Support if a similar message is displayed again.

Pulsing Aborted Charging Timed Out

A charging time limit of 20 seconds is provided for circuit safety. If the capacitors are not charged to the pre-set voltage level after 20 seconds, the "PULSING ABORTED CHARGING TIMED OUT" message is displayed. For assistance with this situation, please contact BTX Technical Support. Press the encoder or the pulse switch once to get back to the Set Parameters screen.

Appendix E: Experimental Troubleshooting

Arcing

Verify electrical component functionality. Verify properties of cell sample (do cells need to be washed? Is the buffer appropriate for application?). Verify properties of transfectant/molecule (Is the DNA well purified?) Try reducing the voltage or increase sample volume until arcing is no longer a problem.

Low (or no) transfection efficiency, or incorporation

Verify physical, biological, and chemical parameters. Verify delivery of the pulse and pulse parameters. Is the voltage correct? Chamber gap? Pulse length or appropriate instrument settings? Number of pulses? If so, follow Optimization Guidelines outlined in Appendix A.

Low viability

Verify physical, biological, and chemical parameters. Are the voltage, chamber gap, pulse length (time constant), pulse number and other instrument settings correct? If so, reduce voltage, pulse length, or number of pulses and re-optimize protocol to improve viability as outlined in Appendix A.

Appendix F: Glossary of Electrical Terms

Amplitude

The instantaneous value of current or voltage in amperes or volts.

Capacitor

A device that stores electric energy in the form of an internal electric field. Energy is delivered when a current flows out of a capacitor. The current normally follows an exponential curve.

Dielectric

A material that has a high resistivity and can store energy in the form of an electric field.

Direct Current (DC)

Current whose amplitude is constant with time. Direct currents are used to form temporary pores in bi-lipid membranes. Cells may fuse when pores in the membranes of two juxtaposed cells reseal after a DC application.

Divergence

The deviation of electric field lines from a parallel homogeneous condition. A highly divergent field has field lines that rapidly change amplitude (or strength) and direction in the area of interest.

Electric Field

The electric potential difference between two points divided by the distance separating those points. Expressed in volt/cm.

Electric Field Force

The mechanical force acting on any electric charge when placed in an electric field.

Exponential Decay

Non linear waveform typical of capacitor charge and discharge currents and voltages. The exponential decay waveform is characterized by its time constant, the time it takes the voltage to decay to $1/e$ of the peak voltage.

Field Strength

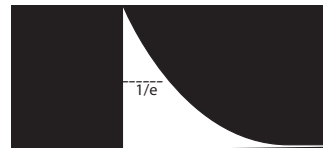
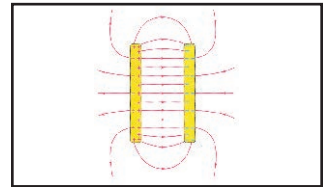
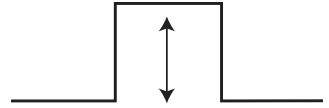
See Electric Field.

Frequency

The number of times an oscillation goes through a complete cycle in one second. The unit is either cycle/sec or (Hz).

Homogenous Electric Field

An electric field where the direction and strength of the field lines are constant.



Appendix G: Glossary of Biological & Technical Terms

Chambers

Electroporation and Electrofusion Chambers are the devices used to hold the cells/molecules to be fused/transfected.

Dielectric Breakdown

The reversible breakdown of lipid bilayer membranes as a result of the application of a DC electroporation pulse. Sufficiently high field strength may increase the membrane potential past a critical point leading to the breakdown of the membrane.

Dielectrophoresis

A consequence of cells being exposed to an inhomogeneous or divergent electric field, resulting in their movement toward electrodes, and subsequent alignment or pearl chain formation.

Electroinsertion

The use of electroporation to insert molecules into lipid bilayer membranes.

Electropermeabilization

The use of electroporation to make cells, protoplasts, or liposomes permeable to ions and small molecules in their extracellular environment.

Electroporation

The application of high electric field pulses of short duration to create temporary pores (holes) in the membranes of cells.

Hydrostatic Pressure

The pressure in liquids at rest.

Lipid Bilayer

An assembly of lipid and protein molecules held together by non-covalent interactions. All biological membranes share this common structure.

Osmotic Pressure

The applied pressure required to prevent the flow of solvents of different concentration across a semipermeable membrane.

Pore

A small, mostly transient, opening in a cell wall caused by the application of a brief high electric field pulse.

Pressure Gradient

The difference in pressure between two points in a medium.



Disposable Electroporation Cuvettes Plus™

Appendix G: Glossary of Biological & Technical Terms (Continued)

Protoplasts

The plant cell proper, with the cellulose cell wall removed.

Relaxation Time

The time a system requires to reach equilibrium.

Transfection

The introduction of nucleic acids into animal cells. Stable transfections result in integration of nucleic acids into host chromosomes and the inheritance of associated traits in progeny cells. Transient transfections result in temporary expression of exogenous nucleic acids.

Transformation

The introduction of nucleic acids into microorganisms and plant cells.

Turgor Pressure

The pressure in capillaries.

Appendix H: Electroporation Pulse Generator Compatibility

Certain components of BTX Electroporation are compatible with components of competitive systems.

Please contact BTX Technical Support for details.

Appendix I: Recommended Reading

Eberhard Neumann, Editor, *Electroporation and Electrofusion in Cell Biology*, Plenum Publishing Corporation, 1989

Michael Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, 199

Donald Chang, Editor-in-Chief, *Guide to Electroporation and Electrofusion*, Academic Press, 1992

Jac A. Nickoloff, Editor, *Electroporation Protocols for Microorganisms*, in *Methods in Molecular Biology*, Vol 47, Humana Press, 1995

Jac A. Nickoloff, Editor, *Animal Cell Electroporation and Electrofusion Protocols*, in *Methods in Molecular Biology*, Vol 48, Humana Press, 1995

Jac A. Nickoloff, Editor, *Plant Cell Electroporation and Electrofusion Protocols*, in *Methods in Molecular Biology*, Vol 55, Humana Press, 1995

For further references regarding specific applications and optimization, please contact BTX Technical Support:

BTX-Division of Harvard Apparatus

84 October Hill Road

Holliston, MA 01746

Phone: 1-508-893-8999

Toll Free: 1-800-272-2775

Fax: 1-508-429-5732

Email: techsupport.btx@harvardapparatus.com

Website: www.btxonline.com

Appendix J: Accessories and Replacement Parts

Catalog No.	Model	Description
MA1 45-0001	6300	ECM® 630 Electroporation System
MA1 45-0051	630	ECM® 630 Electroporator only
MA1 45-0207	630B	Electroporation Safety Stand
MA1 45-0124	610	Disposable Electroporation Cuvettes Plus, 1mm, 50 per bag
MA1 45-0125	620	Disposable Electroporation Cuvettes Plus, 2 mm, 50 per bag
MA1 45-0126	640	Disposable Electroporation Cuvettes Plus, 4 mm, 50 per bag
MA1 45-0400	HT100	Manual 96-Well Plate Handler
MA1 45-0450	HT-P96-2	Disposable 96-Well Plate 2mm gap
MA1 45-0452	HT-P96-4	96-Well Disposable Plate 4mm
MA1 45-0463	HT-P25-P4	25-Well Disposable Plate 4mm pkg 6
MA1 45-0462	HT-P25-4	25-Well Disposable Plate 4mm
MA1 45-0465		25 Well-Adapter HT
MA1 45-0466	HT-P25-2	25-Well Disposable plate 2mm gap
MA1 45-0467	HT-P25-2P	25-Well Disposable Plates 2mm gap pkg 6
MA1 45-0059	Enhancer 3000®	Enhancer 3000® Electroporation Monitoring System

Appendix K: General Care and Cleaning

General Care

Do not store or leave the instrument where the LCD display will be exposed to direct sunlight for long periods of time.



CAUTION

To avoid damage to the instrument, do not expose to sprays, liquids, or solvents.

Cleaning

Inspect the instrument, as often as operating conditions require. To clean the instrument exterior, perform the following steps:

1. Remove loose dust on the outside of the instrument with a lint-free cloth. Use care to avoid scratching the clear plastic display filter.
2. Use a soft cloth dampened with water to clean the instrument. Use an aqueous solution of 75% isopropyl alcohol for more efficient cleaning.



CAUTION

To avoid damage to the surface of the instrument, do not use any abrasive or chemical cleaning agents. Use caution not to drop or cause any unwarranted physical harm to the instrument during any cleaning operations.

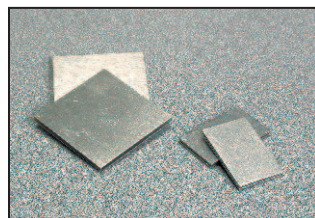
Appendix L: Connecting Electrodes

Cuvettes/Safety Stand

1. Remove the cuvette and the transfer pipette from their sterile packaging.
2. Remove the cuvette cover and fill the cuvette with sample using the transfer pipette and replace cover.
3. Place cuvette on ice for cooling purposes.
4. Push the banana plugs of the Safety Stand into the voltage output of the BTX generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000.
5. Open the Safety Stand cover.
6. Use the thumb wheel to slide the electrodes open.
7. Place a BTX cuvette in between the electrodes with the aluminum of the cuvette coming in contact with the electrodes
8. Secure the cuvette in place by closing the gap with the thumb wheel. The cuvette should fit snugly between the electrodes; however it should be loose enough that it can be pulled out without adjusting the thumb wheel again.
9. Following instructions for the BTX generator. Set the appropriate parameters.
10. Deliver the electroporation pulse (s) to the sample.
Warning: Use proper eye protection during electroporation.
11. Remove cuvette cover and extract the cell solution.
12. Dispose of cuvette and prepare for the next experiment.

Flat Pack Chambers

1. Fill the flat pack chamber with sample using a pipette.
2. Push the banana plugs of the Safety Stand into the voltage output of the BTX generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000.
3. Open the Safety Stand cover.
4. Use the thumb wheel to slide the electrodes open.



Appendix L: Connecting Electrodes (Continued)

5. Place the Flat Pack Chamber in between the electrodes with the aluminum coming in contact with the electrodes.
6. Secure the Flat Pack Chamber in place by closing the gap with the thumb wheel. The Flat Pack Chamber should be snugly placed between the electrodes; however it should be loose enough that it can be pulled out without adjusting the thumb wheel again.
7. Following instructions for the BTX generator, set the appropriate parameters.
8. Deliver the electroporation pulse (s) to the sample.
Warning: Use proper eye protection during electroporation.
9. Remove Flat Pack Chamber and extract the cell solution.
10. Dispose of Flat Pack Chamber and prepare for the next experiment.

Flat Electrodes

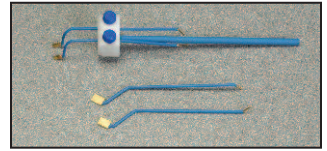
1. Fill the sterile Flat Electrode Chamber using a syringe.
2. Replace the clear plastic tip so the holes in the top align with the holes in the electrode bars.
3. Push the banana plugs at the opposite end of the Flat Electrode (Catalog number 45-0217) into the voltage output of the BTX generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000.
4. Following instructions for the BTX generator, set the appropriate parameters.
5. Deliver the electroporation pulse (s) to the sample.
Warning: Use proper eye protection during electroporation.
6. Extract the cell solution. (The chamber can be rested on ice for cooling purposes)
7. Clean as appropriate and prepare for the next experiment.



Appendix L: Connecting Electrodes (Continued)

Genetrodes/Genepaddles

1. Using the Model 515 Genetrodes holder, loosen the two plastic screws and separate the top half of the positioning plate from the holder. Place the pair of electrodes in the predetermined slots based on the necessary gap size. The electrodes must extend from the holder in the opposite direction of the holder handle. Secure the electrodes by reassembling the holder and tightening the two screws.
2. Attached the micrograbber cable (catalog number 45-0216) to the electrode leads of the Genetrodes/Genepaddles.
3. Push the banana plugs at the opposite end of the micrograbber cable into the voltage output of the BTX generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000.
4. Prepare tissue and sample for electroporation.
5. Following instructions for the BTX generator, set the appropriate parameters.
6. Place the Genetrodes/Genepaddles on the sample using a micromanipulator or manually position them.
7. Deliver the electroporation pulse (s) to the sample.
Warning: Use proper eye protection during electroporation.
8. Remove the electrodes carefully, clean as appropriate and prepare for the next experiment.



Tweezerrodes

1. Attach the Model 524 Tweezerrode Cables to the electrode base of the Tweezerrode.
2. Push the banana plugs at the opposite end of the tweezerrode cable into the voltage output of the BTX generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000. Then plug the banana cable into the voltage output of the BTX Generator.
3. Following instructions for the BTX generator, set the appropriate parameters.



Appendix L: Connecting Electrodes (Continued)

4. Prepare tissue and sample for electroporation. Grasp the tissue between the Tweezerrode electrodes and measure the interelectrode distance. Adjust generator settings if necessary. Inject the sample into the tissue.
5. Deliver the electroporation pulse (s) to the sample.
Warning: Use proper eye protection during electroporation.
6. Remove the tissue carefully, clean as appropriate and prepare for the next experiment.

2-Needle Array

1. Grasping Model 530 or Model 532 2-Needle Array Handle, position the handle over a Model 531 or Model 533 2 Needle Array Assembly and push to secure the 2-needle array to the handle.
2. Push the banana plugs at the opposite end of the 2-Needle array handle into the voltage output of the BTX generator.
Warning: Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000. Then plug the banana cable into the voltage output of the BTX Generator.
3. Following instructions for the BTX generator, set the appropriate parameters.
4. Prepare tissue and sample for electroporation. Apply sample to tissue just before electroporation.
5. Remove the safety shield protecting the needles, place into the tissue, and deliver the electroporation pulse(s).
Warning: Use proper eye protection during electroporation.
6. Discard the 2-needle array and prepare for the next experiment.

Microslides

1. Push the banana plugs at the opposite end of the micrograbber cable (catalog number 45-0216) into the voltage output of the BTX generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000.



Appendix L: Connecting Electrodes

(Continued)

2. Attach the Micrograbbers onto the terminal pins of the Meander Chamber slide. Polarity is not important. Tape the cable to the microscope stage to act as a strain relief and to avoid movement of the slide and its wires.
3. Pipette one drop of cell suspension and reagents to the Microslides/Meander Chamber field.
4. Following instructions for the BTX generator and set the appropriate parameters.
5. Deliver the electroporation pulse (s) to the sample.
Warning: Use proper eye protection during electroporation.
6. Remove the microslides/meander fusion chamber carefully and prepare for the next experiment.

Petri Dish Electrode

1. Plug the HV cables from the Petri Dish Electrode into the voltage output of the BTX Generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000.
2. Prepare sample for electroporation a 35mm Petri Dish.
3. Place the electrode in the 35mm Petri Dish. Allow it to gently rest on the surface of the dish.
4. Following instructions for the BTX generator, set the appropriate parameters. Deliver the electroporation pulse(s) to the sample. **Warning:** Use proper eye protection during electroporation.
5. Remove the Petri Dish Electrode carefully; clean as appropriate and prepare for next experiment.

Petri Pulser

1. Plug the HV cables from the Petri Pulser into the voltage output of the BTX Generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000.
2. Prepare sample for electroporation in 6-well plate or in 35mm Petri Dish.

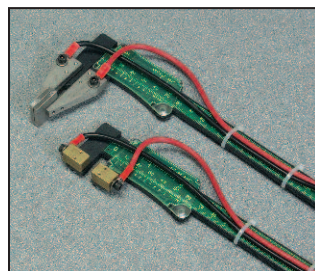


Appendix L: Connecting Electrodes (Continued)

3. Place the electrode in one well of the 6-well plate or 35mm Petri Dish. Allow it to gently rest on the surface of the dish.
4. Following instructions for the BTX generator and set the appropriate parameters.
5. Deliver the electroporation pulse (s) to the sample.
Warning: Use proper eye protection during electroporation.
6. Remove the Petri Pulser carefully; clean as appropriate and complete delivery of pulses to all wells if using a 6-well plate.

Caliper Electrodes

1. Attach the Caliper Electrodes directly to the voltage output ports of the BTX Generator. **Warning:** Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000.
2. Prepare tissue for electroporation.
3. Following instructions for the BTX generator, set the appropriate parameters.
4. Use the Caliper Electrodes to span the target tissue.
5. Deliver the electroporation pulse (s) to the sample.
Warning: Use proper eye protection during electroporation. Do not exceed 500V.
6. Remove the Caliper Electrodes carefully; clean as appropriate and prepare for next experiment.



Appendix M: Plate Handler

Model HT-100 Plate Handler

1. Select a plate size

- a. Select 4mm or 2mm gap plate. For example if currently using 4 mm cuvettes use a 4 mm plate
- i. The plate size should be selected according to the electric field desired, the desired volume of the sample, and the capabilities of the electroporator.

2. Load Plate with Cells

- a. Each column should be loaded with the same number of samples if possible.
- i. Load unused wells in a column with the same sample media at the same volume.

3. Connect the Model HT-100 to the color coded banana cable taking care to match the color of the cable to the color ring around the connector, and then plug the banana cable into the voltage output of the electroporator again matching the color polarity.

4. Place plate (HT 96) or the adapter frame containing plate (HT 25) onto the plate handler so the plate matches the nest plate outline on the handler.

5. Close HT-100 Plate Handler lid firmly to latch. The front panel latch pops out when securely closed.

- a. When the lid is closed correctly, the LED for column 1 should flash.

6. Select appropriate column (1 – 12) to begin electroporation by using the column adjust buttons.

- a. The unit will default to column 1 when lid is closed.
- b. Pressing and holding the button allows the unit to rapidly advance through columns after a short pause.

Appendix M: Plate Handler (Continued)

7. Configure the electroporator with appropriate settings for voltage, resistance and time capacitance
 - a. Set the electroporator for the total number of pulses per well
 - b. Press pulse on the electroporator to initiate the pulse(s) for that column, the plate handler will beep.
 - c. The column LEDs will flash to indicate the active column. Once a column has been electroporated the LED remains steady until the cover is opened.
NOTE: Once the cover is opened the memory of pulsed columns is wiped out.
 - d. Press the column select button to switch to the next column.

Plate handlers may be used with both types of HT plates (HT 96 and HT 25). The following will outline the steps needed to configure the unit for the type of plate being used.



BTX[®]

HARVARD APPARATUS

The Electroporation Experts

84 October Hill Road • Holliston MA, 01746

Phone: **508.893.8999**

Toll Free: **800.272.2775 (U.S. Only)**

Fax: **508.429.5732**

E-mail: **techsupport.btx@harvardapparatus.com**

Web: **www.btxonline.com**