



# **Gemini** Twin Waveform Electroporation Systems Users's Manual



HARVARD APPARATUS

**BTX**<sup>®</sup>

[www.btxonline.com](http://www.btxonline.com)



## Gemini Twin Waveform Electroportation Systems

### RESEARCH ONLY

#### Harvard Apparatus BTX

84 October Hill Rd  
Holliston, MA 01746, USA

Phone: 1-508-893-8999

Fax: 1-800-429-5732

Web: [www.btxonline.com](http://www.btxonline.com)

### Warranty

Harvard Apparatus warrants the BTX Gemini X<sup>2</sup> & BTX Gemini SC<sup>2</sup> Twin Waveform Electroporation Systems for a period of two years from the date of purchase. At its option, Harvard Apparatus BTX 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 HARVARD APPARATUS BTX without HARVARD APPARATUS BTX express and prior approval, (c) used in violation of instructions furnished by HARVARD APPARATUS BTX. This warranty extends only to the original customer purchaser. IN NO EVENT SHALL HARVARD APPARATUS BTX 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, HARVARD APPARATUS BTX 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 BTX Gemini X<sup>2</sup> & BTX Gemini SC<sup>2</sup> instrument is changed in any way from its original factory design or if repairs are attempted without written authorization by HARVARD APPARATUS BTX. Warranty is void if parts, connections or electrodes not manufactured by HARVARD APPARATUS BTX are used with the BTX Gemini X<sup>2</sup> & BTX Gemini SC<sup>2</sup> instrument. If a defect arises within the warranty period, promptly contact Harvard Apparatus BTX, 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](mailto: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.

These instruments contain a high voltage power supply adjustable to 3,000 V. High voltage power supplies present a serious risk of personal injury if not used in accordance with design and/or use specifications, if used in applications on products for which they are not intended or designed, or if they are used by untrained or unqualified personnel.

- The user must read this manual carefully before the instruments are placed into operation.
- Removing the cover will void the warranty.
- Do not connect or disconnect the high voltage cable with the high voltage enabled.
- To connect or disconnect the cable, turn line power off and unplug line (mains) cord.
- Do not touch the electrode tip while the waveforms are being applied

If there are any questions about the operation of this instrument, call Harvard Apparatus BTX Customer service at 1-800-272-2775, or 1-508-893-8999.

### Out of Warranty Service

Proceed exactly as for Warranty Service above. If our service department can assist you by phone or other correspondence, we will be glad to help 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 Harvard Apparatus BTX 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 Harvard Apparatus BTX technicians.

### Repair Facilities and Parts

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

### Caution Notice

The BTX Gemini X<sup>2</sup> & BTX Gemini SC<sup>2</sup> systems are intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) intended for laboratory equipment applications.

The BTX Gemini X<sup>2</sup> & BTX Gemini SC<sup>2</sup> systems comply with the applicable sections of IEC 61010-1:2012 3rd Ed – Safety requirements for electrical equipment for measurement, control, and laboratory use. This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.



## Table of Contents

SUBJECT	PAGE
Safety Information.....	4
Generator Specifications .....	5-6
Introduction.....	7
Electroporation Overview.....	8
Glossary of Terms .....	9
Product Overview .....	10
Unpacking the System.....	11
Operating the System.....	12-13
Setting Time/Date.....	12
Setting Audible Alarm Preferences.....	12
Setting Backlight Preferences.....	13
Displaying Device Information .....	13
Performing Experiments.....	14-25
New Protocols.....	14-19
Preset Protocols.....	19-20
Customizing a Preset Protocol .....	20-21
Using an HT Plate Handler .....	21-23
Deleting a Protocol.....	24
Password Protecting a Protocol.....	25
Touchscreen Button Reference .....	26
Error Messages & Troubleshooting .....	27
Managing Gemini X <sup>2</sup> Series Protocols .....	28
Protocol Manager .....	29-31
Installation .....	29
Overview .....	29
Upload– Generator to PC.....	29
Download– PC to Generator.....	30
Sending Commands to Generator.....	31
Upload Log Files.....	31
Maintenance .....	32
Upgrading BTX Gemini Series Software .....	33-35
USB Virtual Commport Driver Installation.....	33-34
Device Updater Program.....	35
Troubleshooting .....	36
General Optimization Guide for Electroporation .....	37-38
Wave Forms .....	37
Field Strength.....	37
Pulse Length.....	38
Number of Pulses .....	38
Electroporation Buffer .....	38
DNA/RNA Concentrations.....	38
Applications .....	39-41
Mammalian Cell Transfection.....	39
In Vivo, In Utero, In Ovo.....	39
Bacteria and Yeast Transformation.....	39
Plant and Insect Transfection.....	39
References.....	39
General References.....	39-41
Electroporation Buffers .....	42
Experiment Troubleshooting .....	43
Ordering Information .....	44-45
Declaration of Conformity.....	46

## Safety Information

Please read the following safety precautions to ensure proper use of your generator. If the equipment is used in a manner not specified, the protection provided by the equipment may be impaired.

### To Prevent Hazard or Injury

#### Use Proper Line Cord

Use only the specified line cord for this product and make sure line cord is certified for country of use. The operating voltage range for the BTX Gemini Twin Wave Series is 100-240 vac, 50-60 Hz.

#### 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 any connections to the input or output terminals of the product, ensure that the product is properly grounded.

#### Make Proper Connections

Make sure all connections are made properly and securely. Any signal wire connections to the unit must be no longer than 3 meters.

#### Observe All Terminal Ratings

Review the operating manual to learn the ratings on all connections.

#### Use Proper Fuse

Use only specified fuses with product.

#### Avoid Exposed Circuitry

Do not touch any electronic circuitry inside of the product.

#### Do Not Operate with Suspected Failures

If damage is suspected on or to the product do not operate the product. Contact qualified service personnel to perform inspection.

#### Orient the Equipment Properly

Do not orient the equipment so that it is difficult to operate the disconnection device.

#### Place Product in Proper Environment

Review the operating manual for guidelines for proper operating environments.

#### Do Not Operate in Low Impedance Sample or Load

If the electroporation samples have an impedance of less than 20  $\Omega$  in LV and 40  $\Omega$  in HV, this may result in sample loss and potential damage to the unit.

#### Observe All Warning Labels on Product

Read all labels on product to ensure proper usage.



Caution



Protective Ground  
Terminal



Caution Risk of  
Electric Shock



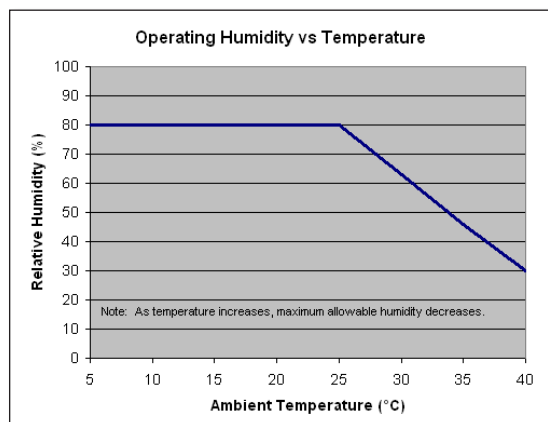
## Generator Specifications

Generator Specifications (V7)		BTX Gemini SC <sup>2</sup>	BTX Gemini X <sup>2</sup>
Square Wave Pulse Ranges			
Voltage Range			
LV Mode	10 to 500 / 5 V res	5 to 500 / 1 V res	
HV Mode	510 to 3000 / 10 V res	505 to 3000 / 5 V res	
Voltage Accuracy			
LV Mode	5%	5%	
HV Mode	5%	5%	
Pulse Length Range			
LV Mode	50 μs to 10 ms / 50 μs res, 10 ms to 100 ms / 1 ms res	10 μs to 999 μs / 1 μs res, 1 ms to 999 ms / 1 ms res	
HV Mode	50 μs to 5 ms / 50 μs res	10 μs to 600 μs / 1 μs	
Multiple Pulsing			
LV Mode	1 to 10	1 to 120 (10 per sample)*	
HV Mode	1 to 2	1 to 36 (3 per sample)*	
Pulse Interval	LV : 0.1S to 10S	0.1 s to 10 sec	
Capacitance in μF			
LV Mode	3775 μF	3775 μF	
HV Mode	85 μF	85 μF	
Voltage Droop	20% Load < 1 K Ω, 10% Load > 1 K Ω	20% Load < 1 K Ω, 10% Load > 1 K Ω	
Exponential Decay Pulse Ranges			
Voltage Range			
LV Mode	10 to 500 / 5 V res	5 to 500 / 1 V res	
HV Mode	510 to 3000 / 10 V res	505 to 3000 / 5 V res	
Voltage Accuracy			
LV Mode	5%	5%	
HV Mode	5%	5%	
Time Constant Range			
LV Mode	1.25 ms to 3.275 S / 1.25 ms	1 ms to 5.158 S	
HV Mode	0.5 ms to 50 ms / 0.5 ms	0.5 ms to 133.875 ms	
Multiple Pulsing			
Multiple Pulsing	No	1 to 12 (Rint < 100 ohm)*, 1 to 24 (Rint > 100 ohm)*	
Pulse Interval	N/A	5 to 30 sec	
Capacitance in μF			
LV Mode	25 to 3275 μF / 25 μF res	25 to 3275 μF / 25 μF res	
HV Mode	10, 25, 50 μF	10, 25, 35, 50, 60, 75, 85 μF	
Internal Resistance (in parallel with load)			
All Modes	50 to 1000 Ω / 50 Ω res	LV 25 to 1575 Ω / 25 Ω res, HV 50 to 1575 Ω / 25 Ω res	
Sample Resistance (Load) Exponential Decay or Square			
LV Mode	Load ≥ 10 Ω	PL ≤ 100ms, load ≥ 8-9 Ω, PL > 100ms, load ≥ 100 Ω	
HV Mode	510-2500 Load ≥ 20 Ω, 2500 to 3000 V, Load ≥ 600 Ω	Load ≥ 40 Ω	
Other Specifications			
Charging Time	LV < 7 sec, HV < 4 sec	LV < 7 sec, HV < 4 sec	
Monitoring	Pulse Voltage, Width, Droop % and Sample Resistance	Pulse Voltage, Width, Droop % and Sample Resistance	
Display	4.3 in color display	4.3 in color display	
Controls	Touch Screen	Touch Screen	
Programmability	Storage over 1000 Protocols	Storage over 1000 Protocols	
Arc Control	Yes	Yes	

\* When used in Plate Handler mode

## Generator Specifications

Generator Specifications (V7)	BTX Gemini SC <sup>2</sup>	BTX Gemini X <sup>2</sup>
<b>Other Specifications (continued)</b>		
Interlock Switches	Yes	Yes
Pre-Pulse Sample resistance check	Yes	Yes
Pulse Over Current Protection	Yes	Yes
PC control	No	Yes
PC communications	No	Yes
Log Report	No	Yes
Remote Operation	No	Yes
Foot Switch Control	No	Yes
Power Ratings	35 W idle and 350 W pulsing	35W idle and 350 W pulsing
Input Voltage ratings	100 to 240 VAC	100 to 240 VAC
CE, ETL Marking, CB Scheme, EuRoHS	Yes	Yes
Dimensions (H x W x D) inches	12.75 x 11.25 x 8.5	12.75 x 11.25 x 8.5
Weight	~ 16 lb	~ 16 lb
Warranty	1 year	2 years
<b>Atmospheric Specifications</b>		
Operating Temperature	4°C to 40°C (40°F to 104°F)	
Storage Temperature	-10°C to 70°C (14°F to 158°F)	
Operating Humidity	See Chart Below	
Storage Humidity	20% to 80% RH, non condensing	
<b>Mode of Operation</b>	Continuous	
<b>Classification</b>	Class I	
<b>Pollution</b>	Degree I	
<b>Installation</b>	Category II	
<b>Supplier Name</b>	Harvard Apparatus BTX	
<b>Supplier Address</b>	84 October Hill Rd., Holliston, MA 01746	
<b>Supplier Phone Number</b>	508-893-8999	
<b>Regulatory Certifications</b>	CE, ETL (UL, CSA), WEEE, EU RoHS & CB Scheme	
<b>Safety Declarations</b>	ANSI/UL 61010-1:2012 Ed. 3 Rev. 2012 ; CAN/CSA C22.2 No. 61010-1:2012 Ed. 3 ; IEC 61010-1:2001 Ed. 2 Corrigendum 1:2002, Corrigendum 2:2003 ; CENELEC EN 61010-1:2001 ; CB Scheme	
<b>EMC Declarations</b>	IEC 61326-1 Ed. 1	
<b>FCC</b>	47 CFR Part 15	







## A Message from Harvard Apparatus BTX

### **Thank you for investing in a Harvard Apparatus BTX Gemini System.**

Since its founding in 1983, BTX's main focus has been in the area of applying controlled electric fields for genetic engineering applications. Because of this, we quickly established a reputation as the technological leader in the fields of electroporation and electrofusion. Our systems have been installed in many prestigious institutes around the globe where they are used successfully for high efficiency transfection, transformation and cell fusion applications. We offer a variety of wave forms, electrodes and chamber options to provide, you, the researchers, with the tools to achieve your goals.

We are vested in your success. To that end, the Harvard Apparatus BTX technical support team constantly tracks published literature for any reference to electroporation and electrofusion. We extract the pertinent experimental conditions and yields from these papers to help us in our efforts to help you. In addition to tracking publications, we are available to you for support at any time for advice in experimental design, product recommendations, troubleshooting, and any other relevant technical advice.

We thank you again for your investment and we look forward to assisting you in any way we can.

Finally, please read this manual carefully before attempting to operate the electroporation system. If you have any questions about the unit or about particular applications, please contact us:

#### **Harvard Apparatus BTX**

84 October Hill Rd  
Holliston, MA 01746 USA

Toll Free: 1-800-272-2775

International Callers: 508-893-8999

Fax: 508-429-5732

Web: [www.btxonline.com](http://www.btxonline.com)

Email: [techsupport.btx@harvardapparatus.com](mailto:techsupport.btx@harvardapparatus.com)

For any customers outside the US or Canada, please call your local Harvard Apparatus BTX dealer or call us directly.

**HARVARD APPARATUS**

**BTX<sup>®</sup>**



## Electroporation Overview

### DNA Delivery Into Cells Using Electroporation

#### General Electroporation Discussion

Electroporation is the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane. Their presence allows molecules, ions, and water to pass from one side of the membrane to the other. When the electric field is applied the ions inside and outside the cell membrane migrate. As the charge builds up on either side of the membrane the membrane weakens and the pathways form permitting material outside of the cell to enter. If the electric field is promptly removed the pathways close and the membrane reseals. If the electric field duration is too long the pathways increase and the cell is killed. Efficient electroporation depends on proper selection of electric field waveforms. The electropores are located primarily on the membrane areas which are closest to the electrodes. The pathways form in about a microsecond and seal in seconds to minutes. The duration of the electric field is tens of microseconds to tens of milliseconds.

The use of electroporation was described by Neumann in the early 1980's. The routine use of electroporation became very popular with researchers through the 1980's because it was found to be a practical way to place drugs, or other molecules into cells. In the late 1980's, scientists began to use electroporation for applications in multi-cellular tissue.

In the early 1990's Lluís Mir of the Institute Gustave-Roussy was the first to use electroporation in a human trial to treat external tumors.

Research has shown that the induction of pathways is affected by three major factors. First, cell-to-cell biological variability causes some cells to be more sensitive to electroporation than other cells. Second, for pathways to be induced, the product of the pulse amplitude and the pulse duration has to be above a lower limit threshold. Third, the number of pathways and effective pathway diameter increases with the product of "amplitude" and "duration." Although other factors are involved, this threshold is now understood to be largely dependent on a fourth factor, the reciprocal of cell size. If the upper limit threshold is reached pore diameter and total pore area are too large for the cell to repair by any spontaneous or biological process, the result is irreversible damage to the cell or cell lysis. Because the mechanism of electroporation is not well understood, the development of protocols for a particular application has usually been achieved empirically, by adjusting pulse parameters (amplitude, duration, number, and inter-pulse interval).

Research shows that certain experimental conditions and parameters of electrical pulses may be capable of causing many more molecules to move per unit time than simple diffusion. There is also good evidence (Sukharev et al., 1992) that DNA movement is in the opposite direction.

An additional important consideration is when the voltage pulse is applied to the cells and medium that the amount of current that flows is dependent on the conductivity of the material in which the cells are located. Some material is quite conductive and severe heating will occur if the pulse duration is too long. Therefore long duration fields will kill cells by destroying the membrane and heating.

The electric field in which the cells are located is produced by two system components. The first is the voltage waveform generator and the second is the electrode which converts the voltage into the electric field.

As the charge accumulates at the membrane, which is a capacitance, the voltage across the membrane increases.

$$\text{voltage} = \text{capacitance charge}$$

As charge accumulates at the membrane, the voltage across the membrane increases. Neumann et al. (1989) described the equation that relates the transmembrane voltage (TMV) to electric field intensity:

$$\text{TMV}^{3/2} = E r |\cos \alpha|$$

where:

**E = electric field intensity in volts/cm**

**r = the cell radius in cm**

**$\alpha$  = angle off the center line**

Pores in the membrane will begin to form as the voltage increases from its quiescent value of a few tenths of a volt to more than 0.5 volts. To produce a TMV of 1 volt across the membrane of a cell with 7  $\mu\text{m}$  radius, the required electric field intensity is:

$$E = \frac{2}{3} * \frac{1}{7 \times 10^{-4}} = 950 \text{ volts/cm}$$

The number of pores and effective pore diameter increase as the product of pulse amplitude and duration increase. At the upper limit threshold, pore diameter and total pore area become too large for the cell to repair by any spontaneous or biological process. The result is irreversible damage to the cell or cell lysis.

Another important point to consider is the generation of heat during electroporation. Heat production is directly related to current intensity which is, in turn, dependent on the conductivity of the material through which the electric field is applied. Standard saline solutions such as PBS and many tissue culture media are highly conductive and, thus, will generate considerable amounts of heat when used in cell electroporation. Excessive heating can be detrimental to cell viability. The effects of heating can be reduced by using a low conductivity medium such as BTX's Cytoporation medium to resuspend cells prior to electroporation.

Although electroporation is an effective method for introducing macromolecules onto cells, the biological mechanisms by which cells become electroporated are not completely understood. Therefore, the development of specific protocols for particular applications is usually achieved by empirical adjustment of pulse parameters (i.e. amplitude, duration, pulse number, and interpulse interval).



## Glossary of Terms

**Capacitance** – The ability of a body to store an electrical charge. Any object that can be electrically charged exhibits capacitance.

**Capacitor** – A device that stores energy in the form of an electric field. A capacitor consists of two metal plates insulated from each other by a dielectric (insulating, usually a plastic material such as Mylar) material. In an ideal capacitor, no conduction current flows between the plates after the capacitor is completely charged. Capacitors can be fixed, variable, or adjustable.

**Cell Form** – The format in which cells present for experimentation. Cell forms include suspension, in vivo, in ovo, ex plant, adherent, whole organism, etc.

**Dielectric Breakdown** – The reversible breakdown of bi-lipid layer membranes as a result of the application of a DC electroporation pulse. A sufficiently high field strength may increase the membrane potential past a critical point leading to the breakdown of the membrane.

**Dielectric Constant** – For a given dielectric (nonmetallic) material, the ratio of electrical capacitance of a dielectric-filled capacitor to a vacuum capacitor of identical dimensions.

**Divergence** – The deviation of field lines (e.g. electric field lines) from parallel, homogeneous conditions. A highly divergent field is a very inhomogeneous field where the value and direction of the field change drastically in the area under consideration.

**Electrolytic** – A fluid containing charged molecules is called an electrolyte. Electrolytic properties are associated with such a fluid, such as the ability to conduct current.

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

**Electroporation Cuvette** – Square chambers with electrodes on two sides, usually measuring 1 mm, 2 mm or 4 mm in gap, for the purpose of electroporating cells in suspension. The cell type being electroporated typically determine the gap. Prokaryotic cells typically use 1 mm or 2 mm gap cuvettes, while eukaryotic cells will typically use 2 mm or 4 mm gap sizes.

**Exponential Decay Waveform** – This waveform is mainly used for transforming cells during electroporation. In this type of pulse the set voltage is released from the capacitor and decays rapidly and exponentially over time (millisecs). 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 and capacitance settings to achieve a wide pulse gradient.

**Field Strength** – The potential difference between two points (electrodes) (in Volts) divided by the distance between the electrodes (called gap, and expressed in cm). Expressed as V/cm or kV/cm. This is true only if the electric field is homogenous as it is in parallel plate electrodes.

**Gap** – The distance between electrodes.

**Homogenous Electric Field** – The direction and field strength are constant.

**Hydrostatic Pressure** – The pressure in liquids at rest.

**Inhomogeneous Electric Field** – Direction and strength of the electric field vary.

**Number of Pulses** – The number of pulses the sample will be exposed to.

**Osmotic Pressure** – The applied pressure required to prevent the flow of solvents of different concentration across a semi-permeable membrane.

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

**Potential Difference** – The difference (in Volts) between points in an area between electrodes.

**Protocols** – The method for performing an experiment.

**Pressure Gradient** – The difference in pressure between two points in a medium.

**Pulse Interval** – The time between multiple pulses.

**Pulse Length/Pulse Duration** – The length of time an electric signal is applied.

**Specialty Electrodes** – Electrodes used with the BTX Gemini X<sup>2</sup> to perform electroporation on a wide variety of cell forms. Some applications include in vivo, in ovo, in utero, or ex plant electroporation. Harvard Apparatus BTX offer many types of specialty electrodes.

**Square Waveform** – This waveform is typically used for eukaryotic cells. It is characterized by the voltage delivered, the duration of the pulse, the number of pulses and the length of the interval between pulses.

**Time Constant** – (represented by the greek letter tau, T) is the amount of time required for the actual voltage of the delivered pulse to decrease to a value 1/e of the true peak pulse.

**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.

**Voltage** – The difference of electric potential between two electrodes (expressed in volts (V) or kilovolts (kV)).

**Waveforms** – The shape of time-varying electric signals.



## Product Overview

The **BTX Gemini X<sup>2</sup>** is a highly advanced twin waveform generator incorporating both square and exponential decay waves in a single unit. The BTX Gemini X<sup>2</sup> has been designed with these waveform combinations to enable researchers to easily and efficiently electroporate eukaryotic cells and prokaryotic cells in all forms with one easy to use setup. The BTX Gemini X<sup>2</sup>, which can be operated via PC or remote control, boasts a wide range of voltage (5-3000 v, 1 v or 5 v increments), pulse length (10  $\mu$ S – 1S, 1  $\mu$ S resolution), time constant options (which include capacitance choices from 25 to 3275  $\mu$ F in LV and 10, 25, 35, 50, 60, 75, 85  $\mu$ F in HV), along with multiple pulsing options with both the square waveform and the exponential decay waveform, and unparalleled pulse delivery accuracy. The BTX Gemini X<sup>2</sup>, with over 1,000 custom protocol storage, monitors and displays pre-pulse sample resistance as well as delivered voltage values and records logs of all experiment parameters internally, which can be downloaded to a computer for analysis and QC. The generator is controlled through a color LCD touch screen interface and incorporates USB communications. The pulse can also be activated by a foot switch. The enhanced safety features of the BTX Gemini X<sup>2</sup> protect users as well as precious samples. The BTX Gemini X<sup>2</sup> is designed to give researchers the ultimate flexibility in their experiments, making it possible to perform electroporation on tissues and organs in vivo (as well as in utero, in ovo, ex plant) on adherent cells, and cells in suspension in either single cuvettes or 96 well plates. The Safety Dome allows researchers to safely work with electroporation cuvettes, up to two at a time, giving researchers the ability to experiment on sample volumes from 20  $\mu$ l up to 800  $\mu$ l. This state of the art system comes complete with the twin waveform generator for cell electroporation in all forms, dozens of preprogrammed protocols for commonly electroporated cells, Safety Dome, 30 cuvettes, cuvette stand, user manual, two year warranty, unlimited application support and the same high quality researchers have come to expect from Harvard Apparatus BTX.

The **BTX Gemini SC<sup>2</sup>** is a twin waveform generator incorporating both square and exponential decay waves in a single unit. These waveform combinations enable researchers to easily and efficiently electroporate eukaryotic and prokaryotic cells in suspension with one easy to use setup. The BTX Gemini SC<sup>2</sup> boasts a wide range of voltage (10-3000 v, 5 v or 10 v increments), pulse length (50  $\mu$ S – 5 mS, 50  $\mu$ S resolution), time constant options (which include capacitance choices from 25 to 3275  $\mu$ F in LV and 10, 25 or 50  $\mu$ F in HV), multiple pulsing option with the square waveform and unparalleled pulse delivery accuracy. The BTX Gemini SC<sup>2</sup>, with unlimited custom protocol storage, monitors and displays pre-pulse sample resistance as well as delivered voltage values. The generator is controlled through a color LCD touch screen interface. The enhanced safety features of the BTX Gemini SC<sup>2</sup> protect users as well as precious samples. The Safety Dome allows researchers to safely work with electroporation cuvettes, up to two at a time, giving researchers the ability to experiment on sample volumes from 20  $\mu$ l up to 800  $\mu$ l. This affordable system comes complete with the dual waveform generator for suspension cell electroporation, dozens of preprogrammed protocols for commonly electroporated cells, Safety Dome, 30 cuvettes, cuvette stand, user manual, two year warranty, unlimited application support and the same high quality researchers have come to expect from Harvard Apparatus BTX.

### What is the difference between the Gemini X<sup>2</sup> and the Gemini SC<sup>2</sup>?

The Gemini X<sup>2</sup> is designed to give researchers the ultimate control and flexibility in their experiments by making it possible to perform electroporation on tissues and organs in vivo (as well as in utero, in ovo, ex plant) on adherent cells, as well as cells in suspension in either single cuvettes or 96 well plates, can be controlled remotely via footswitch or PC, and it offers storage of pulse data. Because of the Gemini X<sup>2</sup> broad range of use, the specifications are wide-ranging, making the Gemini X<sup>2</sup> the most versatile electroporation system available today.

The Gemini SC<sup>2</sup> is designed for researchers working to electroporate cells in suspension in cuvettes. This system cannot accommodate multiple pulsing with the exponential decay waveform, remote operation, specialty electrodes or 96 well options. For this reason, some of the specifications of the Gemini SC<sup>2</sup> are not as extensive as what is available in the Gemini X<sup>2</sup>.

## Unpacking the System

The shipping carton in which your BTX Gemini Twin Waveform Electroporation System is packed has been specifically designed to provide maximum protection to the instrument during transportation and normal handling conditions. Upon receipt, the carton should be examined for any external damage resulting from shipment.

Open the carton and carefully remove the BTX Gemini Twin Waveform Electroporator and inspect the unit for any apparent damage. Save the carton and packing materials for future transportation and shipping requirements.

### Packing Data

Check the packing slip to ensure that all items ordered and listed are included in the shipment. Inform Harvard Apparatus BTX immediately if any parts are missing or damaged.

### Power Source

As received, the instrument is ready for use with either 100-240 V AC, 50/60 HZ.

The power requirements are 350 watts. In the USA, the power cord has a standard three prong plug.

### Installation

Once you have determined that the components of the system have not sustained any obvious damage in shipment, proceed with the installation. The location of the BTX Gemini Twin Waveform Electroporator should be a dry, level, sturdy surface free from extremes in ambient temperature, dust or chemical exposures. Unpack the safety dome, cuvette rack and disposable cuvette chambers.

Connect the safety dome or in the case of the BTX Gemini X<sup>2</sup> system, specialty electrodes or HT plate handler, into the connectors at the bottom right-hand side of front panel.

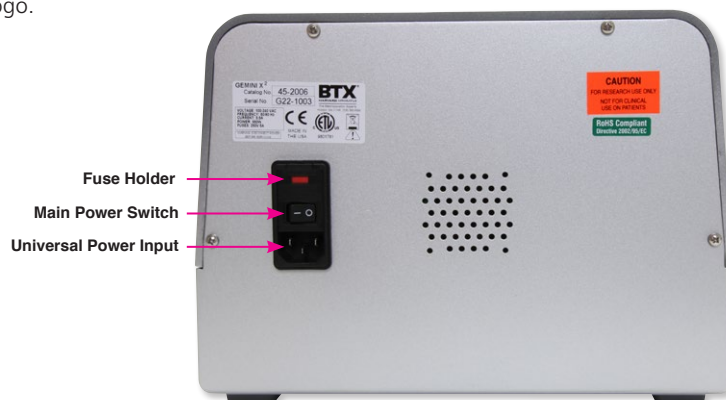
Connect the mains/power cord to into the back panel at the bottom left.

Power up the system by pushing the rocker switch located on the back panel at the bottom left. The display will flash the BTX logo. Once the software initializes, the Home screen will appear.

You are now ready to begin your work.



Gemini SC<sup>2</sup>

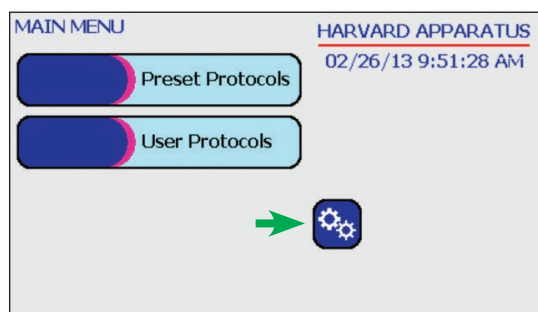


## Operating the System

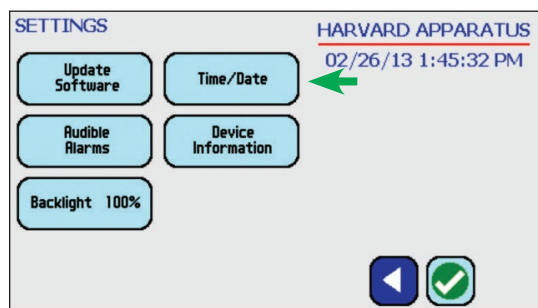
### Setting Time/Date

After initializing, the home screen will appear.

Tap the Gears icon



Tap Time/Date icon.



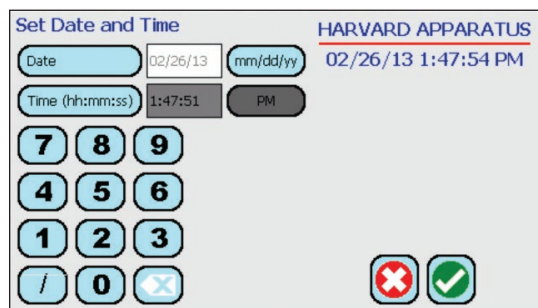
Tap Date icon and use the keypad to type the date.

Tap Time icon and use the keypad to type the time.

Tap the Green Check icon to save and return to the settings screen.

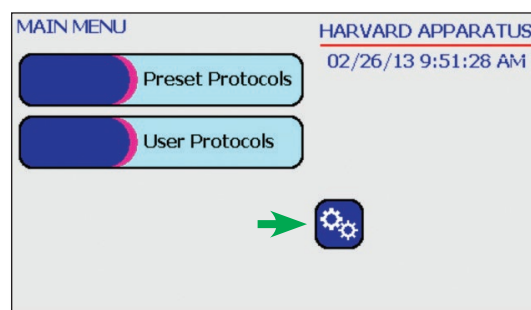


**NOTE:** You may change the date and time format by tapping icons to the right of the given values.

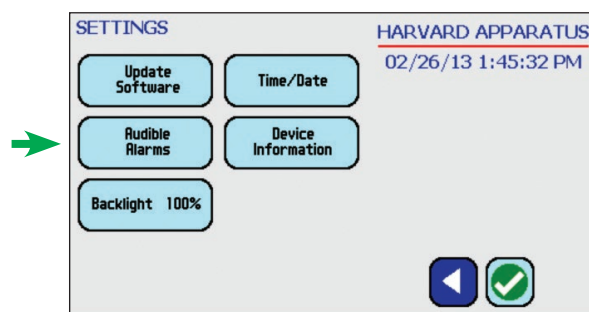


### Setting Audible Alarm Preferences

On the home screen, tap the Gears icon

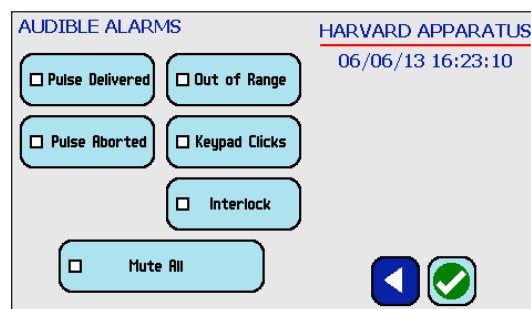


Tap Audible Alarms icon.



Select preferred audible alarms by tapping the icons to activate or deactivate.

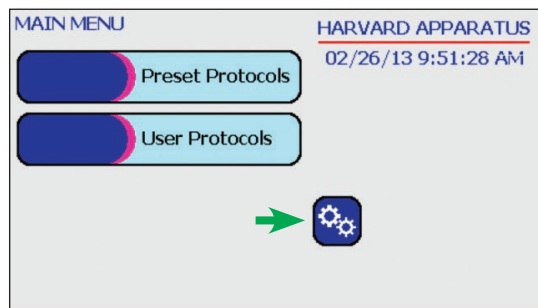
Tap the Green Check icon to save and return to the settings screen.



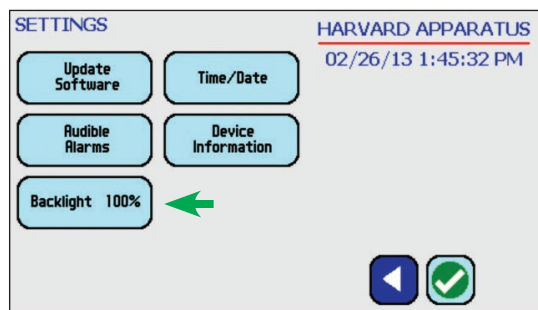
## Operating the System

### Setting Backlight Preferences

On the home screen, tap the Gears icon.

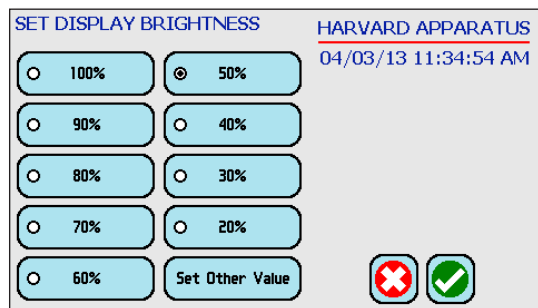


Tap Backlight icon.



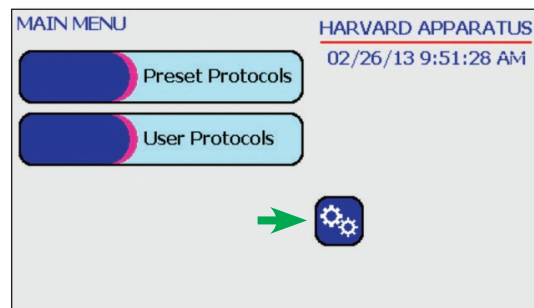
Select preferred brightness by tapping the icons indicating % brightness.

Tap the Green Check icon to save and return to the settings screen.

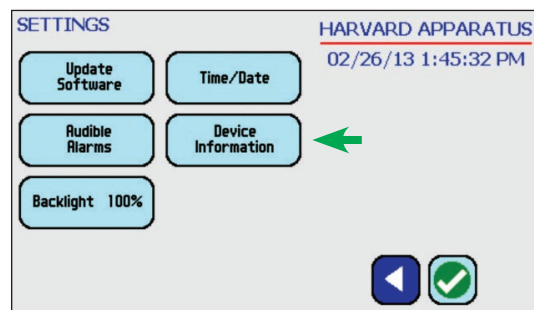


### Displaying Device Information

On the home screen, tap the Gears icon.

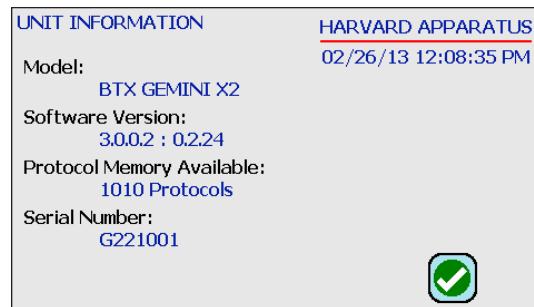


Tap Device Information icon.



View device information.

Tap the Green Check icon to return to the settings screen.

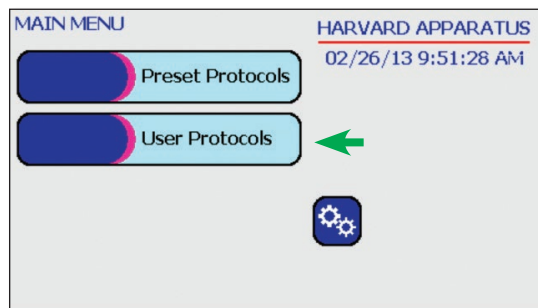




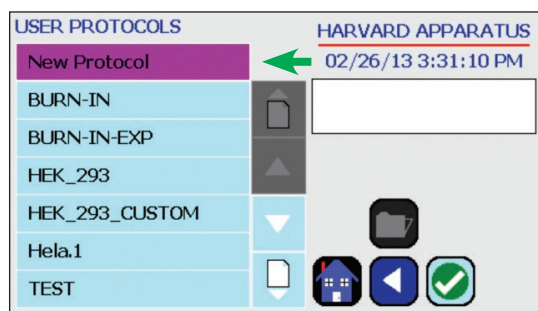
## Performing Experiments

### New Protocols

On the home screen, tap User Protocols icon.

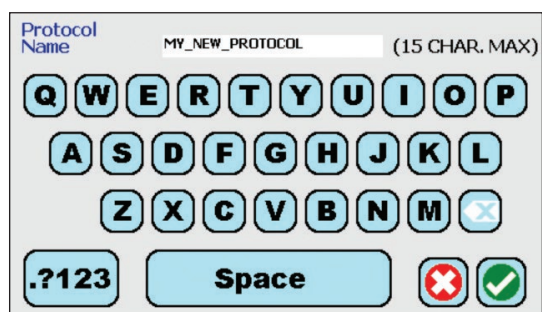


Double tap New Protocol.



Use keyboard to name protocol.

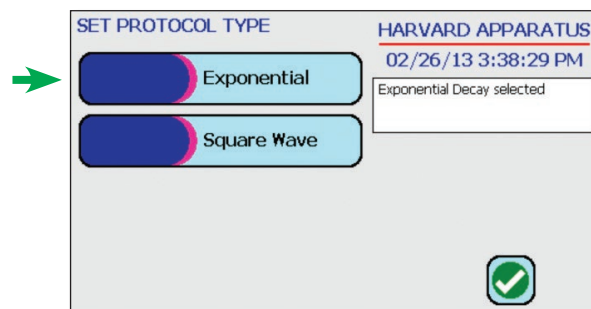
Tap the Green Check icon to save.



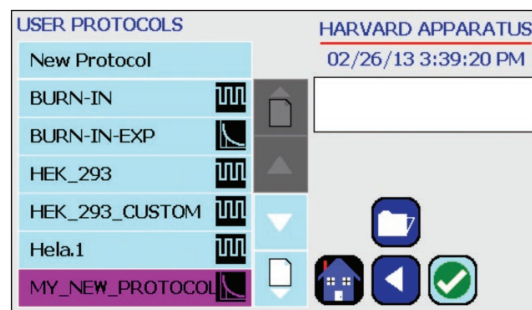
### Exponential Decay Wave

Tap the Exponential icon.

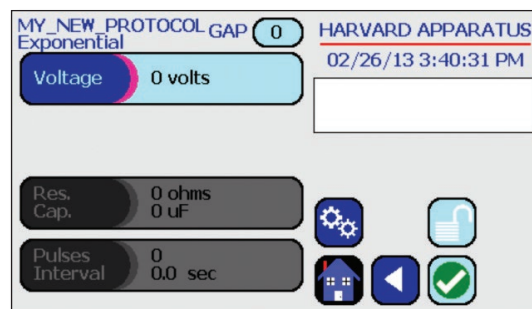
Tap the Green Check icon to save.



Double tap your newly saved protocol.

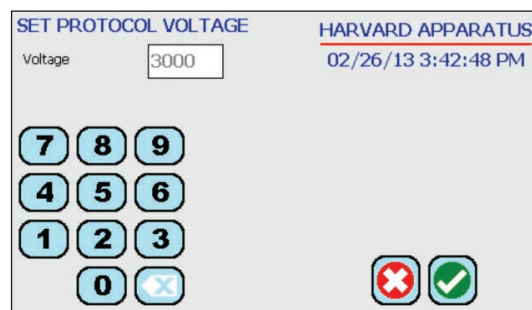


Tap the Voltage icon.



Use the numeric keypad to set voltage.

Tap the Green Check icon to save.



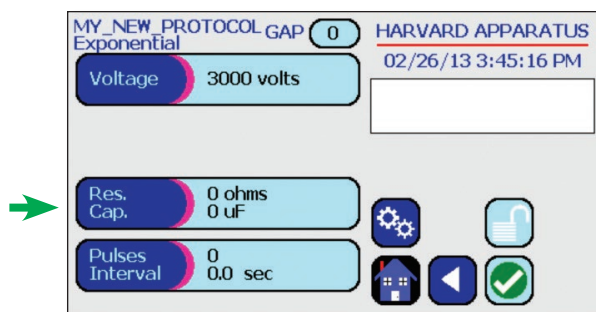


## Performing Experiments

### New Protocols (continued)

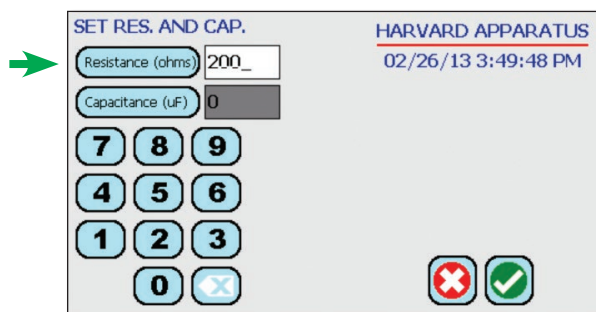
#### Exponential Decay Wave (continued)

Tap the Res. Cap. icon.



Tap the Resistance (ohms) icon.

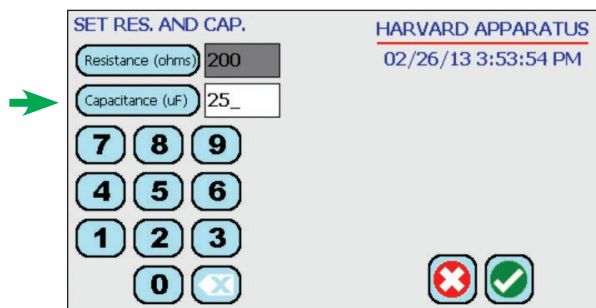
Use the numeric keypad to set resistance value.



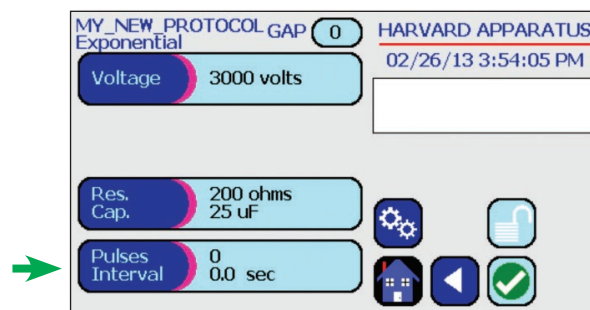
Tap the Capacitance (uF) icon.



**NOTE:** LV and HV mode capacitance values differ. Use the numeric keypad to set resistance value.



Tap the Pulses Interval icon.

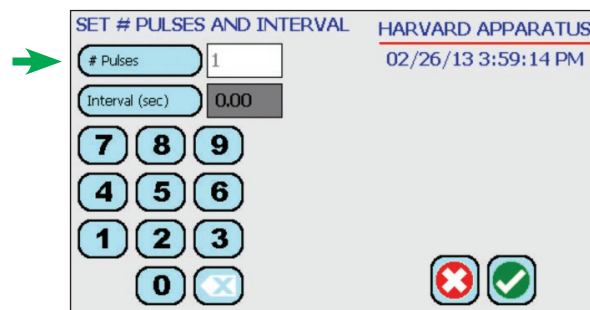


Tap the # Pulses icon.

Use the numeric keypad to set the # of pulses.



**NOTE:** Only the BTX Gemini X<sup>2</sup> can perform multiple pulsing with Exponential Decay Wave, the BTX Gemini SC<sup>2</sup> cannot perform multiple pulsing with Exponential decay waves.



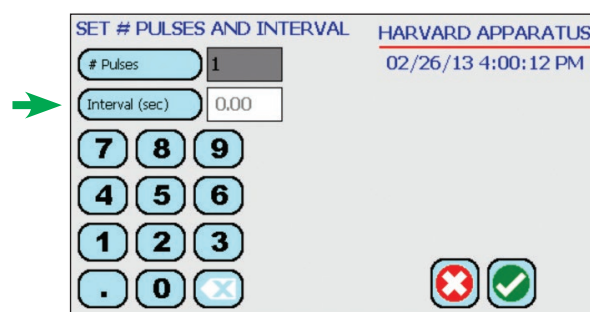
Tap the Interval (sec) icon.

Use the numeric keypad to set the interval between pulses.

Tap the Green Check icon to save.



**NOTE:** Intervals must only be set when performing multiple pulsing.



## Performing Experiments

### New Protocols (continued)

#### Exponential Decay Wave (continued)

Tap the Gap icon.

MY\_NEW\_PROTOCOL GAP 0 HARVARD APPARATUS  
Exponential 02/26/13 3:45:16 PM

Voltage 3000 volts

Res. Cap. 0 ohms 0 uF

Pulses Interval 0 0.0 sec

Use the numeric keypad to set the gap between electrodes.

Tap the Green Check icon to save.

SET PROTOCOL GAP HARVARD APPARATUS  
Gap (mm) 2 02/26/13 4:03:56 PM

7 8 9  
4 5 6  
1 2 3  
. 0

Tap the Green Check icon to proceed to the run screen.

MY\_NEW\_PROTOCOL GAP 2 HARVARD APPARATUS  
Exponential 02/26/13 4:04:58 PM

Voltage 3000 volts

Res. Cap. 200 ohms 25 uF

Pulses Interval 1 0.0 sec

View your protocol.

To run, tap the Omega icon.

RUN PROTOCOL HARVARD APPARATUS  
MY\_NEW\_PROTOCOL Exponential Decay 04/02/13 3:29:11 PM

Set	Meas
Voltage (v)	3000
Time Const. (ms)	5
Resistance (ohm)	200
Capacitance (uF)	25
# of Pulses	1
Pulse Interval (s)	--
Gap width (mm)	2
Meas. Load (ohm)	--

Plate Handler

GO

With load measurement OK, press the Go icon to run protocol.

RUN PROTOCOL HARVARD APPARATUS  
MY\_NEW\_PROTOCOL Exponential Decay 04/02/13 3:31:23 PM

Set	Meas
Voltage (v)	3000
Time Const. (ms)	4.18
Resistance (ohm)	200
Capacitance (uF)	25
# of Pulses	1
Pulse Interval (s)	--
Gap width (mm)	2
Meas. Load (ohm)	OK

Plate Handler

GO

Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs (BTX Gemini X<sup>2</sup> only) for future use.

You may continue pulsing, go back to set-up screen, or to the home screen.



**NOTE:** See the Protocol Manager user manual for instructions on viewing and downloading log data.

RUN PROTOCOL HARVARD APPARATUS  
MY\_NEW\_PROTOCOL Exponential Decay 04/02/13 3:32:58 PM

Set	Meas
Voltage (v)	3000 2940
Time Const. (ms)	4.18 4.4
Resistance (ohm)	200
Capacitance (uF)	25
# of Pulses	1
Pulse Interval (s)	--
Gap width (mm)	2
Meas. Load (ohm)	OK 1012

Pulses delivered = 1  
PRESS TO CLEAR MESSAGE

Plate Handler

GO

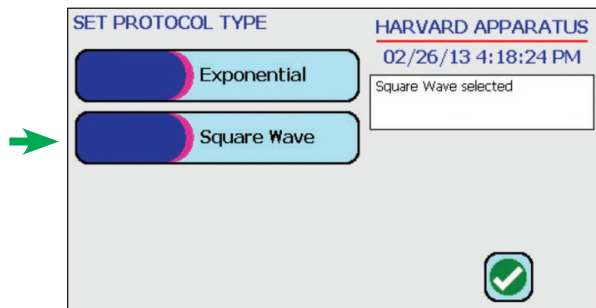
## Performing Experiments

### New Protocols (continued)

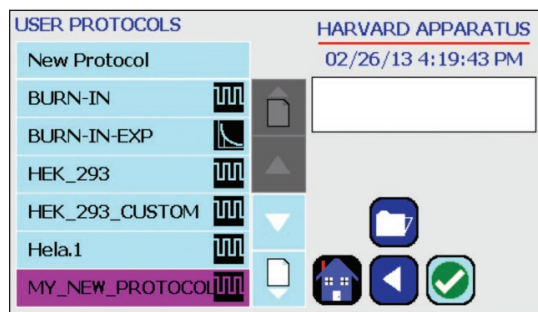
#### Square Wave

Tap the Square Wave icon.

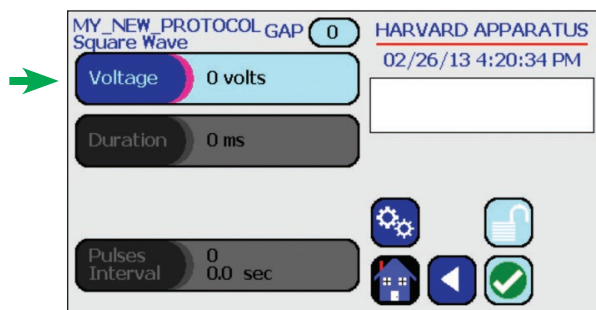
Tap the Green Check icon to save.



Double tap your newly saved protocol.

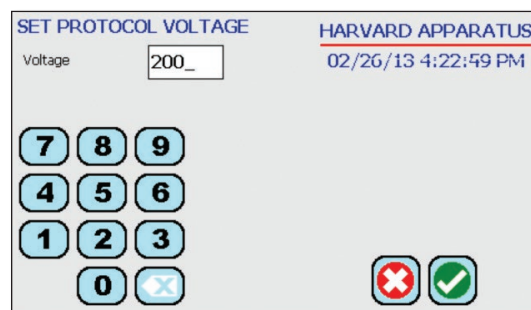


Tap the Voltage icon.

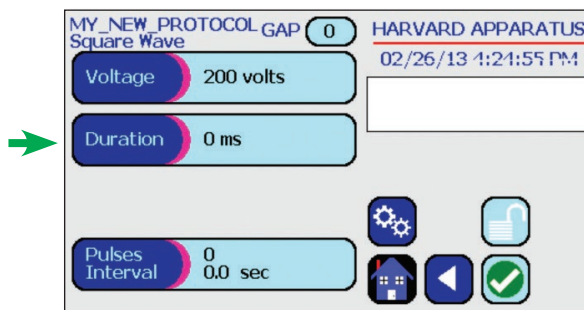


Use the numeric keypad to set voltage

Tap the Green Check icon to save.

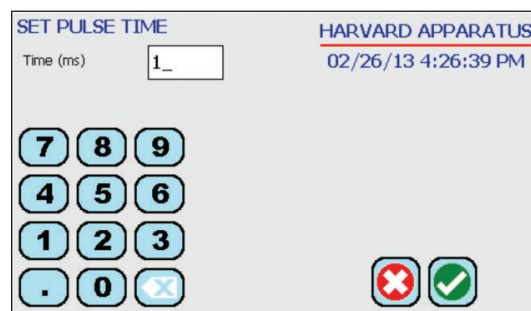


Tap the Duration icon.



Use the numeric keypad to set the pulse length.

Tap the Green Check icon to save.

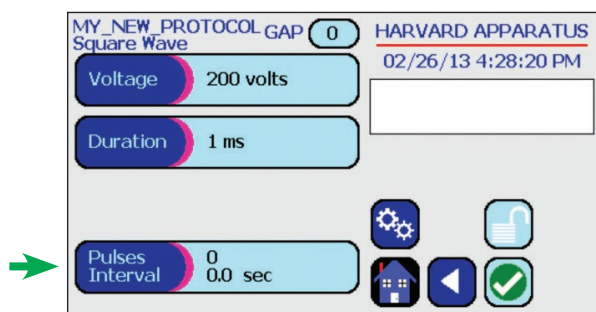


## Performing Experiments

### New Protocols (continued)

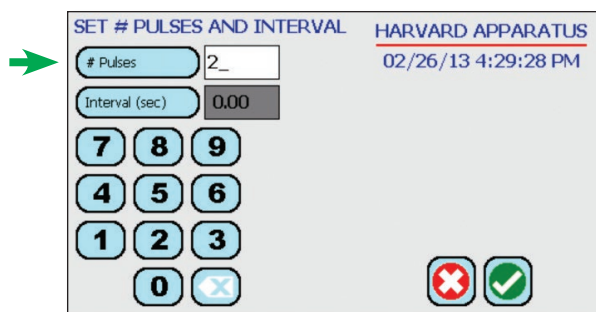
#### Square Wave (continued)

Tap the Pulses Interval icon.



Tap the # Pulses icon.

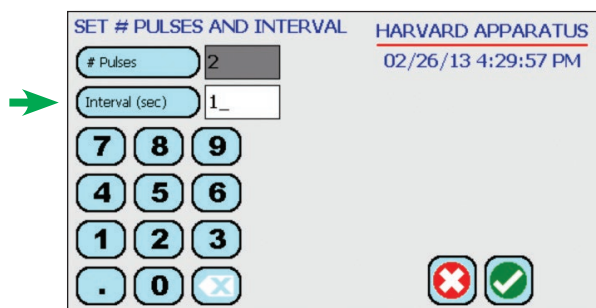
Use the numeric keypad to set the # of pulses.



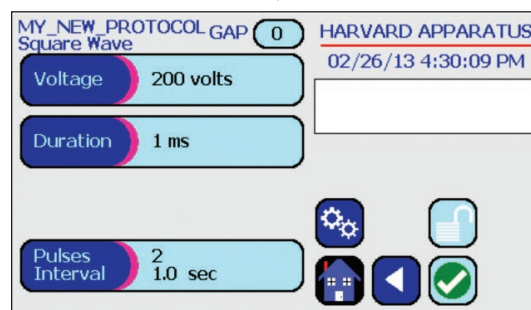
Tap the Interval (sec) icon.

Use the numeric keypad to set the pulse interval.

Tap the Green Check icon to save.

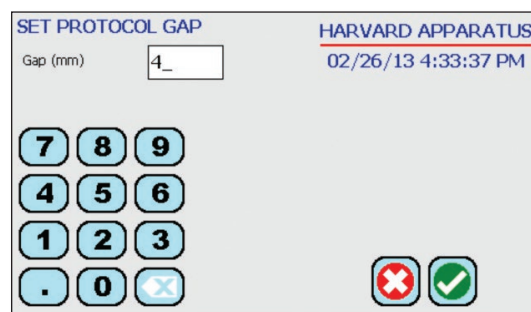


Tap the Gap icon

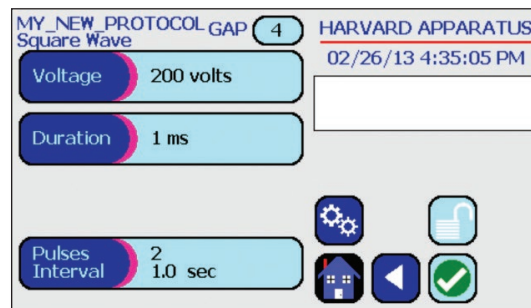


Use the numeric keypad to set the gap between electrodes.

Tap the Green Check icon to save.



Tap the Green Check icon to proceed to the run screen.





## Performing Experiments

### New Protocols (continued)

#### Square Wave (continued)

View your protocol.

To run, tap the Omega icon.

**RUN PROTOCOL** HARVARD APPARATUS  
 MY\_NEW\_PROTOCOL 04/02/13 4:06:40 PM  
 Square Wave Set Meas  
 Voltage (v) 200  
 Duration (ms) 1  
 Resistance (ohm) --  
 Droop % --  
 # of Pulses 2  
 Pulse Interval (s) 1.0  
 Gap width (mm) 4  
 Meas. Load (ohm) --  
 Plate Handler  
 GO

With load measurement OK, press the Go icon to run protocol.

**RUN PROTOCOL** HARVARD APPARATUS  
 MY\_NEW\_PROTOCOL 04/02/13 4:06:58 PM  
 Square Wave Set Meas  
 Voltage (v) 200  
 Duration (ms) 1  
 Resistance (ohm) --  
 Droop % 0.0  
 # of Pulses 2  
 Pulse Interval (s) 1.0  
 Gap width (mm) 4  
 Meas. Load (ohm) OK  
 Plate Handler  
 GO

Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs (BTX Gemini X<sup>2</sup> only) for future use.

You may continue pulsing, go back to the set-up screen, or to the home screen.

**RUN PROTOCOL** HARVARD APPARATUS  
 MY\_NEW\_PROTOCOL 04/02/13 4:07:39 PM  
 Square Wave Set Meas  
 Voltage (v) 200 206  
 Duration (ms) 1 1  
 Resistance (ohm) --  
 Droop % 0.0 0.0  
 # of Pulses 2  
 Pulse Interval (s) 1.0  
 Gap width (mm) 4  
 Meas. Load (ohm) OK 976  
 Plate Handler  
 GO

### Preset Protocols

On the home screen, tap the Preset Protocols icon.

**MAIN MENU** HARVARD APPARATUS  
 02/26/13 9:51:28 AM  
 Preset Protocols  
 User Protocols  
 Settings

Double tap desired cell type.

**PROTOCOL TYPE** HARVARD APPARATUS  
 02/26/13 1:22:11 PM  
 Bacteria / Yeast  
 Mammalian  
 In Vivo  
 Plant  
 Adherent  
 High Throughput

Scroll through available cells.

Double tap desired cells.

**PROTOCOL SELECT** HARVARD APPARATUS  
 Mammalian 22 Protocols 02/26/13 1:24:37 PM  
 ms stem cells  
 rt stem cells  
 CHO  
 K562  
 HEK 293  
 293T

## Performing Experiments

### Preset Protocols (continued)

Review parameters.

Tap Omega icon to measure pre pulse load resistance.

RUN PROTOCOL		HARVARD APPARATUS	
HEK 293 #867		Set	Meas
Square Wave			04/02/13 4:16:48 PM
Voltage (v)	100		
Duration (ms)	10		
Resistance (ohm)	--		
Droop %	--		
# of Pulses	1		
Pulse Interval (s)	--		
Gap width (mm)	4		
Meas. Load (ohm)	--		

With load measurement OK press the Circle icon to run protocol.

RUN PROTOCOL		HARVARD APPARATUS	
HEK 293 #867		Set	Meas
Square Wave			04/02/13 4:17:14 PM
Voltage (v)	100		
Duration (ms)	10		
Resistance (ohm)	--		
Droop %	0.3		
# of Pulses	1		
Pulse Interval (s)	--		
Gap width (mm)	4		
Meas. Load (ohm)	OK		

Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs (BTX Gemini X<sup>2</sup> only) for future use.

You may continue pulsing, go back to the protocol select screen, or to the home screen.

RUN PROTOCOL		HARVARD APPARATUS	
HEK 293 #867		Set	Meas
Square Wave			04/02/13 4:17:49 PM
Voltage (v)	100		
Duration (ms)	10		
Resistance (ohm)	--		
Droop %	0.3		
# of Pulses	1		
Pulse Interval (s)	--		
Gap width (mm)	4		
Meas. Load (ohm)	OK		988

### Customizing a Preset Protocol

On the home screen, tap the Preset Protocols icon.

MAIN MENU		HARVARD APPARATUS	
			02/26/13 9:51:28 AM
Preset Protocols			
User Protocols			

Double tap desired cell type.

PROTOCOL TYPE		HARVARD APPARATUS	
			02/26/13 1:22:11 PM
Bacteria / Yeast			
Mammalian			
In Vivo			
Plant			
Adherent			
High Throughput			

Scroll through available cells.

Tap desired cells.

Tap Folder icon.

PROTOCOL SELECT		HARVARD APPARATUS	
Mammalian 22 Protocols			02/26/13 1:24:37 PM
ms stem cells			
rt stem cells			
CHO			
K562			
HEK 293			
293T			

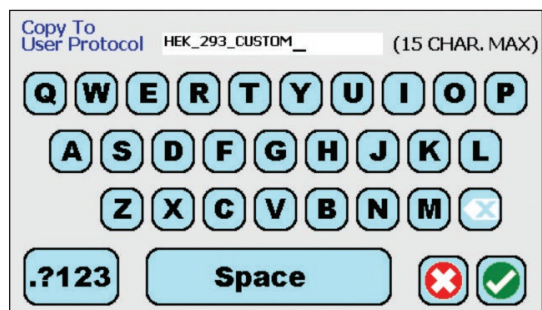


## Performing Experiments

### Customizing a Preset Protocol (continued)

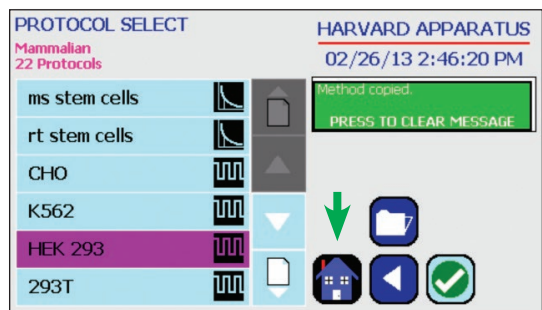
Use keyboard to name copy of protocol.

Tap the Green Check icon to save.

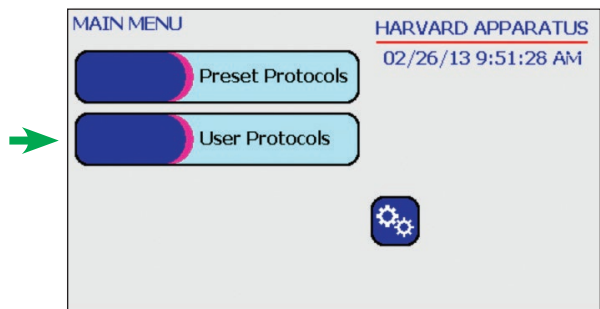


The message board will turn green and alert the user that the method has been copied.

Tap the Home icon.

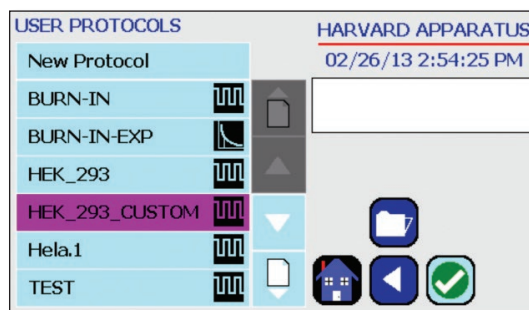


On the home screen, tap User Protocols icon.



Scroll through user protocols to locate the newly saved method.

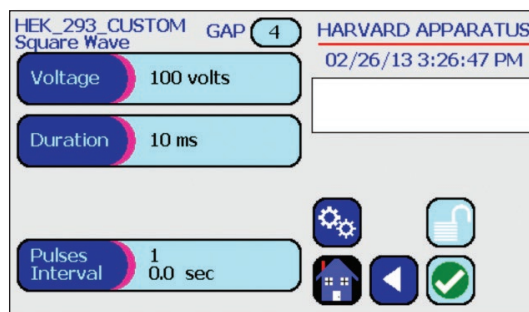
Double tap the newly saved method.



Tap the parameter(s) requiring customization.

Proceed to modify selected parameters.

Tap the Green Check icon to proceed to the run screen.

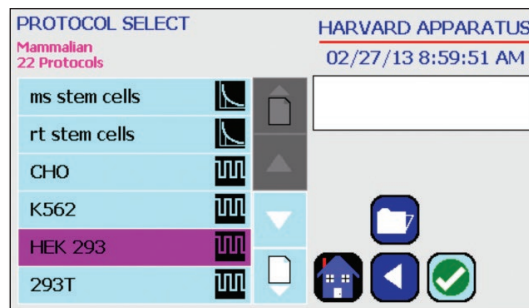


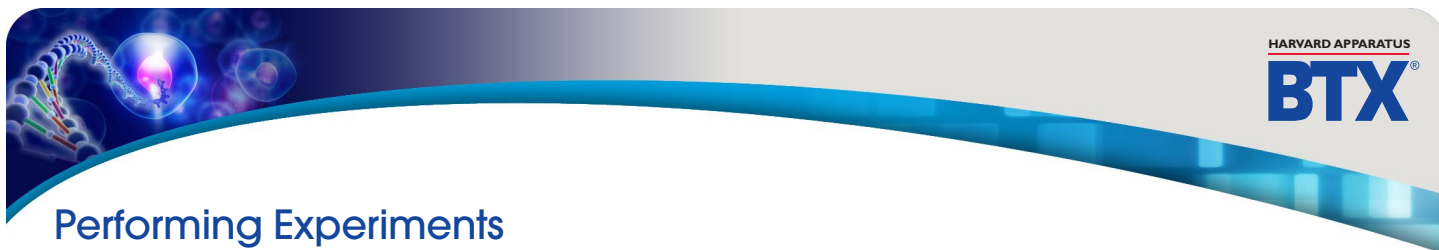
### Using an HT Plate Handler (BTX Gemini X<sup>2</sup> Only)

Plug the HT Plate Handler into the front ports of the BTX Gemini X<sup>2</sup>.

Scroll through available cells.

Double tap desired cells.





## Performing Experiments

### Using an HT Plate Handler (BTX Gemini X<sup>2</sup> Only) (continued)

Review parameters.

Tap Plate Handler icon.

Using the numeric keypad icon, type the number of columns being pulsed.

Tap the Green Check icon to save.



**NOTE:** Note the message box. It will indicate how many pulses should be set in the HT plate handler. This number will be a combination of both pre pulse resistance measurements and DC pulses.

Review parameters.

Set the HT plate handler with the correct number of pre-pulse resistance measurement and DC pulses.

Tap the Go icon to run protocol.

The Square icon can be used to stop the protocol at any time.



**NOTE:** Generator will display readings of the 1st and last pulse of each column.

Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs for future use.

You may continue pulsing, go back to the settings screen or protocol select screen, or to the home screen.

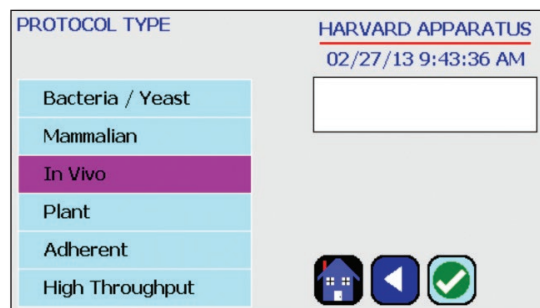
## Performing Experiments

### Using Specialty Electrodes (BTX Gemini X<sup>2</sup> only)

Plug the specialty electrodes into the front ports of the BTX Gemini X<sup>2</sup>

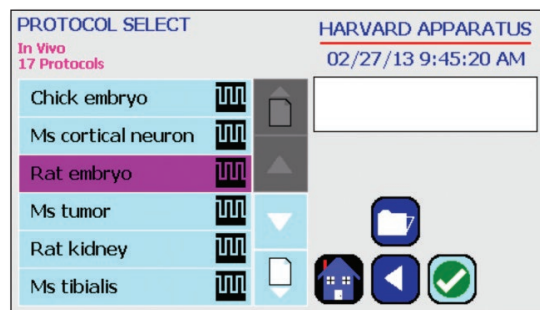
Scroll through available protocols.

Double tap desired selection.



Scroll through available cells.

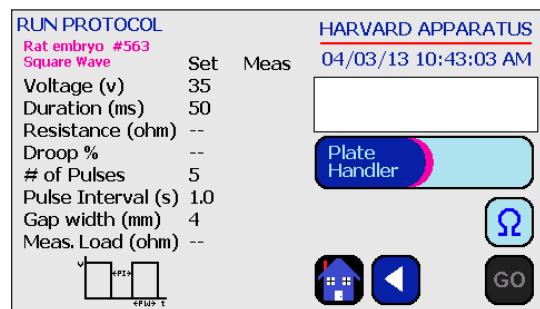
Double tap desired cells.



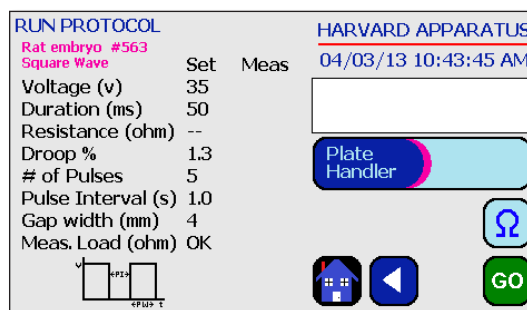
Review parameters.

Position electrodes on area being electroporated.

To run tap the Omega icon.



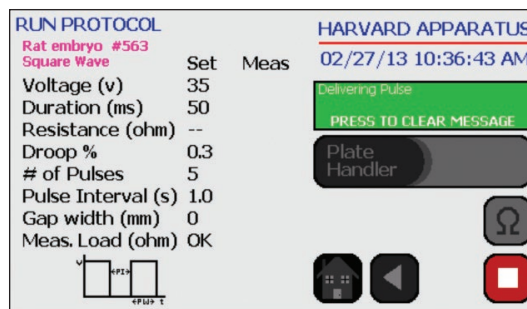
With load measurement OK, press the Go icon to run protocol.



While the pulse is being delivered, the Square icon can be used to stop the protocol at any time.

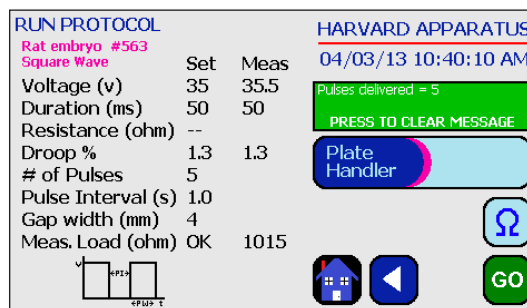


**NOTE:** Generator will display readings after each pulse.



Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs for future use.

You may continue pulsing, go back to the settings screen or the protocol select screen, or to the home screen.



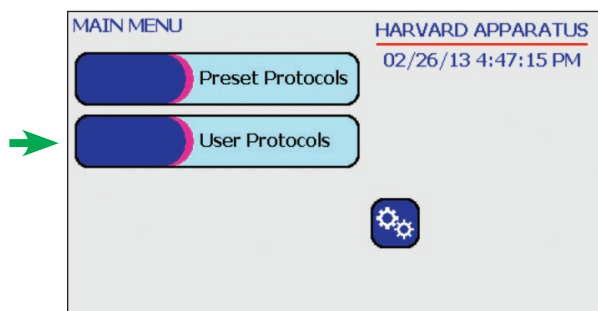
## Performing Experiments

### Deleting a Protocol



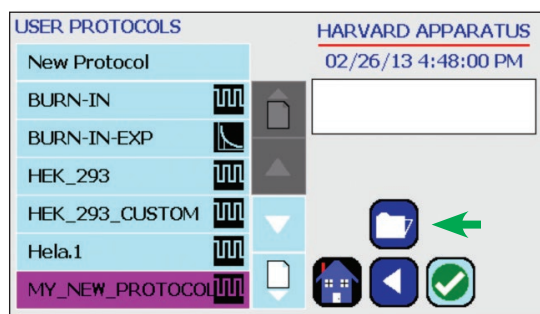
**NOTE:** Preset protocols cannot be deleted.

On the home screen, tap the User Protocols icon.

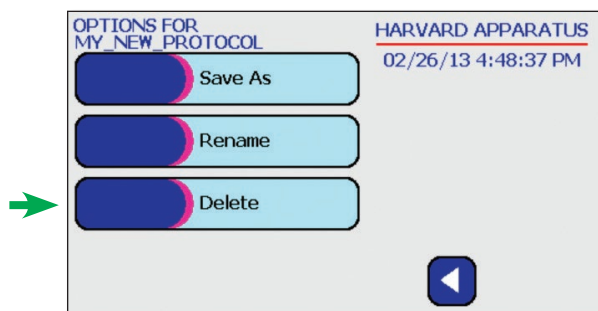


Tap the protocol you wish to delete.

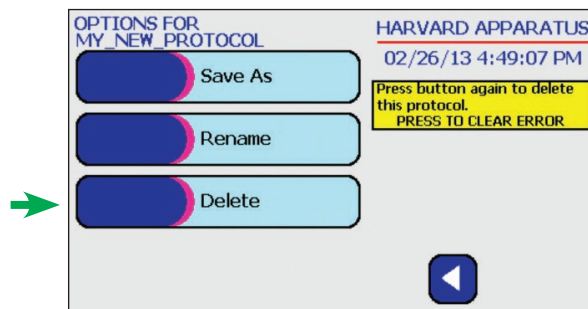
Tap Folder icon.



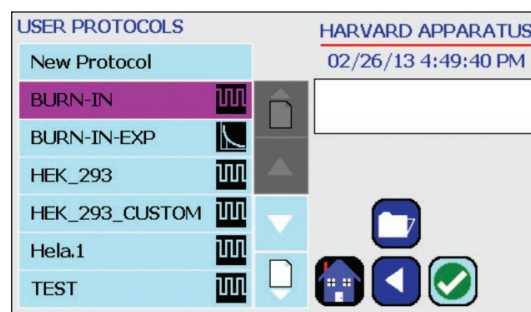
Tap Delete icon.



Confirm delete by tapping the Delete icon again.



Protocol has been removed from the menu.

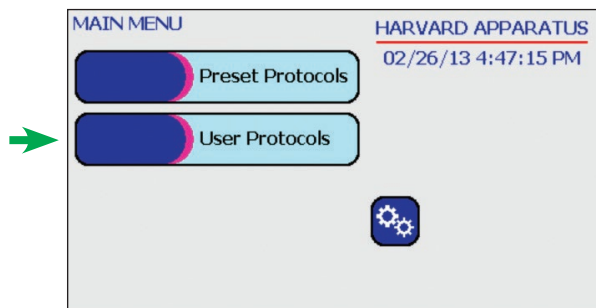




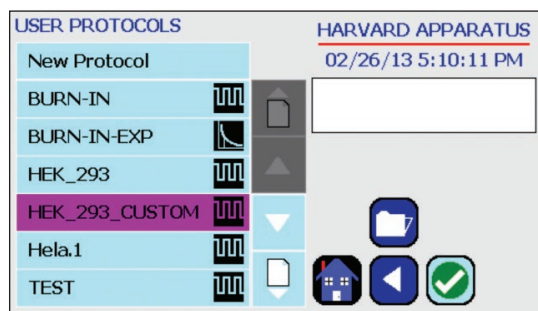
## Performing Experiments

### Password Protecting a Protocol

On the home screen, tap the User Protocols icon.

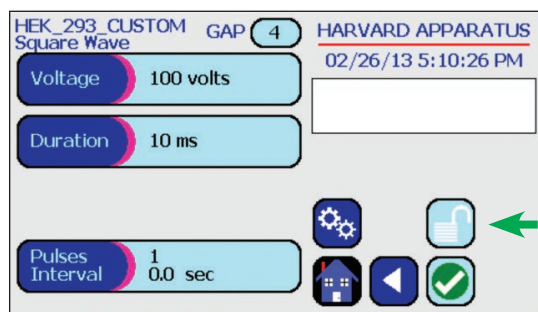


Double tap protocol you wish to password protect.

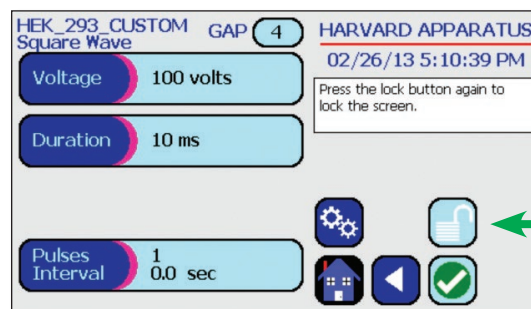


View the protocol.

Tap the Open Lock icon.

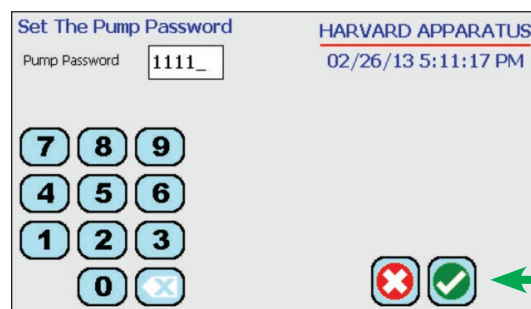


Confirm password protection by tapping the Open Lock icon again.



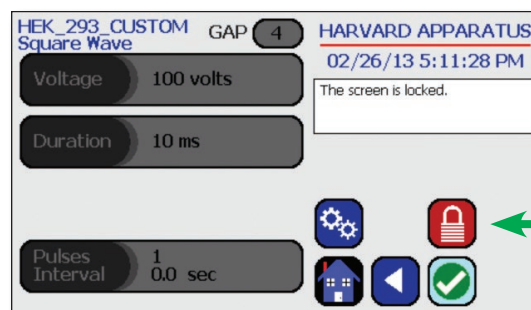
Use the numeric keypad to set the password.

Tap the Green Check icon to save.



The protocol is now locked against modifications.

To Unlock press the lock icon. Type in the password then Tap the Green Check icon.



## Touchscreen Button Reference



### Accept

Used to accept the parameters/settings on a screen and advance to the next screen in the menu, also used in place of a double tap on various icons.



### Back

Used to go back one screen.



### Cancel

Used to cancel any changes/entries on a screen and return to the previous screen in the menu.



### Exponential Decay Wave Indicator

Used to indicate when a protocol utilizes exponential decay wave pulses.



### File Options

Access the File Option menu that is used to save, rename and delete protocols.



### Home/Main Menu

Access Preset Protocols, User Protocols and Settings.



### Page Down

Used to page down in a display list.



### Page Up

Used to page up in a display list.



### Pre-Pulse Resistance Measurement

Used to measure the resistance of the sample prior to delivering the DC pulse.



### Run Protocol

Used to deliver the pulse protocol to the sample.



### Scroll Down

Used to scroll down in a display list.



### Scroll Up

Used to scroll up in a display list.



### Settings

Access the settings menu used to adjust the following parameters: Date and Time, Audible Alarms, Backlighting, and software updates. Displays device information.



### Stop Protocol

Used during the pulse delivery sequence to stop the progress of the protocol.



### Square Wave Indicator

Used to indicate when a protocol utilizes square wave pulses.



### Touch Screen Locked

Indicates that the touch screen is currently locked. Press the icon and enter password to unlock the touch screen.



### Touch Screen Unlocked

Indicates that the touch screen is currently unlocked. Pressing the icon twice will allow the user to password protect the protocol.



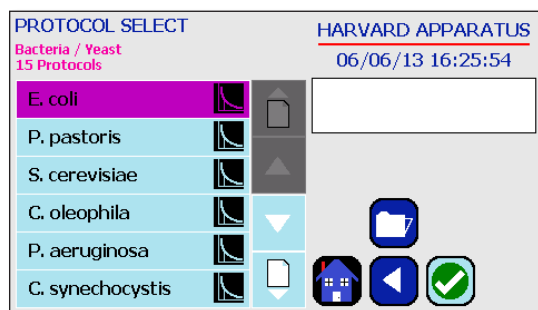


## Error Messages & Troubleshooting

Display Error Message	Error Description	User Troubleshooting
<b>Power Loss Error</b>	Power loss during pulsing.	Power lost while protocol was running.
<b>PFC Over Temp</b>	Pfc module over temperature.	Hardware error. Please contact the manufacturer.
<b>Interlock Open</b>	Electrode interlock open.	Connect electrode connectors to the front of the generator and make sure the cuvette safety dome cover is closed.
<b>IGBT Switch Fault</b>	IGBT switches fault.	Hardware error. Please contact the manufacturer.
<b>Charge Voltage Max Error</b>	Charging voltage above the expected voltage.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
<b>Charge Current Max Error</b>	Charging current above the expected current.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
<b>LV Cap Bank Max</b>	Low voltage capacitor bank voltage above expected value.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
<b>LV Cap Bank Min</b>	Low voltage capacitor bank voltage below expected value.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
<b>HV Cap Bank Max</b>	High voltage capacitor bank voltage above expected value.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
<b>HV Cap Bank Min</b>	High voltage capacitor bank voltage below expected value.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
<b>Charge Time Max</b>	Charging capacitor bank to selected voltage exceeded max time estimated.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
<b>Pulse Droop Error</b>	Pulse voltage droop exceeded estimated droop.	A) Increase the sample resistance. This can be achieved by: 1. Increasing the gap size 2. Reducing the volume 3. Use a less conductive buffer B) Reduce the pulse duration
<b>Pulse Voltage Overshoot</b>	Pulse voltage monitor (p-vmon) pulse overshoot.	Hardware error. Please contact the manufacturer
<b>Pulse Voltage Mon too Low</b>	Pulse voltage monitor (p-vmon) below min voltage.	Hardware error. Please contact the manufacturer
<b>Pulse Voltage Mon too High</b>	Pulse voltage monitor (p-vmon) above max voltage.	Hardware error. Please contact the manufacturer
<b>Pulse Current Mon too Low</b>	Pulse current monitor (p-imon) below min current.	Hardware error. Please contact the manufacturer
<b>Pulse Current Mon too High</b>	Pulse current monitor (p-imon) above max current.	Hardware error. Please contact the manufacturer
<b>Arc_Detected</b>	Arc detected during pulsing.	Arc could happen due to the one or more of the following conditions: 1. Sample resistance is very low for the voltage selected 2. Gap size is too small for the voltage selected 3. Pulse duration is too long
<b>No_HB_from_GUI</b>	Communication error.	Hardware error. Please contact the manufacturer
<b>Sample Resistance Out Of Range</b>	The measured sample load is out of range.	Increase the sample resistance. This can be achieved by 1. Increasing the gap size 2. Reducing the volume 3. Replace the buffer with less conductive type of buffer
<b>Over Current Pulse Abort</b>	Pulse aborted due to pulse over current.	The over current protection feature is added to prevent sample arcing. This could occur when the sample resistance changes during the pulse: 1. Sample resistance is very low for the voltage selected 2. Gap size is too small for the voltage selected 3. Pulse duration is too long
<b>Idle V I Max</b>	Voltage or current in idle state is higher than expected.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
<b>Charge Cap Bank A Max</b>	Charge capacitor bank A voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
<b>Charge Cap Bank A Min</b>	Charge capacitor bank A voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
<b>Charge Cap Bank B Max</b>	Charge capacitor bank B voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
<b>Charge Cap Bank B Min</b>	Charge capacitor bank B voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
<b>Charge Cap Bank C Max</b>	Charge capacitor bank C voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
<b>Charge Cap Bank C Min</b>	Charge capacitor bank C voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
<b>Dump Time Max</b>	The capacitor bank did not fully discharge in the estimated time.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
<b>Invalid Protocol</b>	The generator was requested to run an invalid protocol.	Verify that the used protocol is within the allowed specifications of the generator
<b>System Processing (Busy)</b>	The control command is invalid or was issued while the generator was busy.	Allow more time between events
<b>TC_Error</b>	The measured rc time while delivering an exponential decay pulse is invalid.	Hardware error. Please contact the manufacturer
<b>Pulse Duration Error</b>	The pulse duration did not meet the expected duration.	Hardware error. Please contact the manufacturer
<b>Unit Type Sel Error</b>	The unit hardware and software do not match the correct type.	Hardware error. Please contact the manufacturer
<b>Error: 0X#</b>	Multiple errors.	Multiple error. Please contact the manufacturer

## Managing Gemini X<sup>2</sup> Series Protocols

All Preset and User-Defined Protocols are stored in the Protocol List for ongoing use. Over time, you may want to manage previously created Protocols to keep your Protocol List easy to navigate. In addition, the BTX Gemini X<sup>2</sup> allows you to export Protocol information to an externally connected generator or to a computer that will be used to control operation. This section of the Guide describes the file management facilities available on the BTX Gemini X<sup>2</sup>.



### Saving a Copy of a Protocol

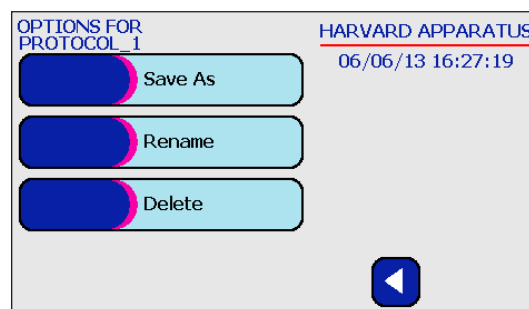
Follow these instructions to save a copy of a Protocol under a new name:

1. From **Protocol Configuration** screen, choose the **Protocol Name** button to enter the **Mode Selection** screen.
2. From the list of available Protocols, select (press once) the Protocol you want to save a copy of, then choose the **File Options** button to enter the File Options screen.
3. Choose **Save As/Copy** to access the **Protocol Name Entry** screen. The current **Protocol Name** will be displayed in the text entry box.
4. On the **Protocol Name** screen, type the new name for your Protocol using the onscreen keyboard. Choose the Symbols/Numbers button (.?123) to display the list of available numbers and symbols that can be included in the Protocol Name. To switch the keyboard back to letters mode, choose the Letters button (ABC). [15 characters maximum]
5. When finished entering the new Protocol Name, choose **Accept** to save your changes and return to the **File Options** screen.
6. Choose **BACK** to return to the Mode Selection screen

### Renaming a Protocol

Follow these instructions to rename a previously saved user protocol:

1. From the **Protocol Configuration** screen, choose the **Protocol Name** button to enter the **Protocol Mode** screen.
2. From the list of available Protocols, select (press once) the Protocol you want to rename, then choose the **File Options** button to enter the **File Options** screen.
3. Choose Rename Protocol to access the **Protocol Name Entry** screen. The current Protocol Name will be displayed in the text entry box.
4. On the **Protocol Name** screen, type the new name for your Protocol using the onscreen keyboard. Choose the Symbols/Numbers button (.?123) to display the list of available numbers and symbols that can be included in the Protocol Name. To switch the keyboard back to letters mode, choose the Letters button (ABC). [15 characters maximum]
5. When finished entering the new Protocol Name, choose **Accept** to save your changes and return to the File Options screen.
6. Choose **Back** to return to the Protocol.



# Protocol Manager

## Installation

Insert the generator CD into the CDROM, a file containing the CD's contents will open automatically. Navigate to the Protol Manager folder, under Applications. Right click on "Protocol Manager". Select "Send To" then "Desktop" to create a shortcut on your desktop.



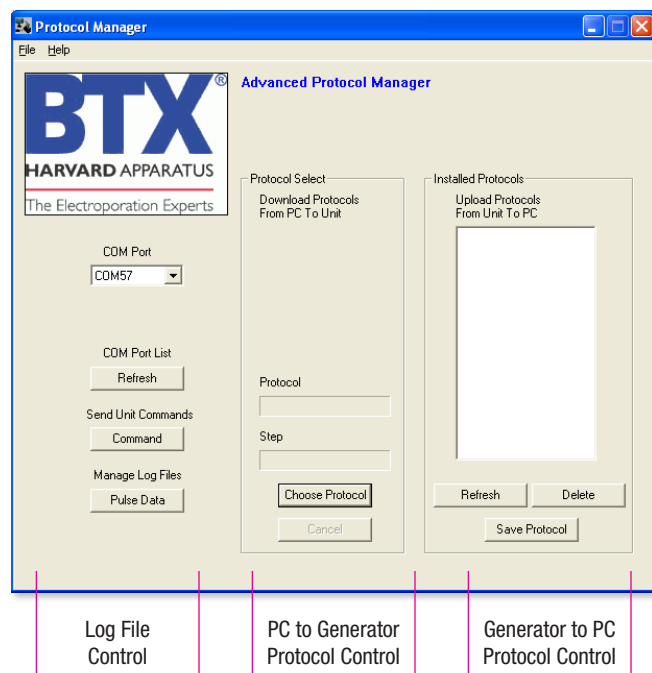
**NOTE:** Compatible with Windows XP & Windows 7.

1. Connect the generator to the PC using a USB cable.
2. Turn generator on.
3. Start Protocol Manager.
4. Select the proper COM port.

## Overview

Protocol Manager allows the user to:

- Upload protocols from the Gemini X<sup>2</sup> generator to a PC.
- Download protocols from PC to the Gemini X<sup>2</sup> generator.
- Send commands to the Gemini X<sup>2</sup> generator using a PC.
- Upload log files from the Gemini X<sup>2</sup> generator to PC.

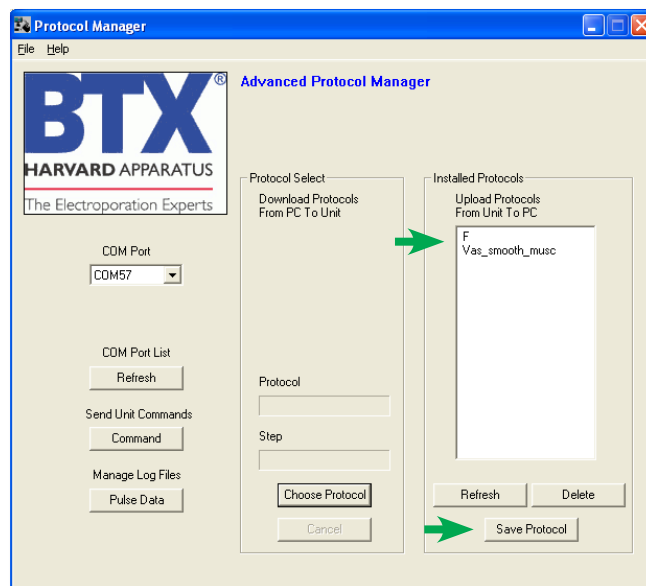


## Upload– Generator to PC

1. Select Protocol(s)
2. Click 'Save Protocol'

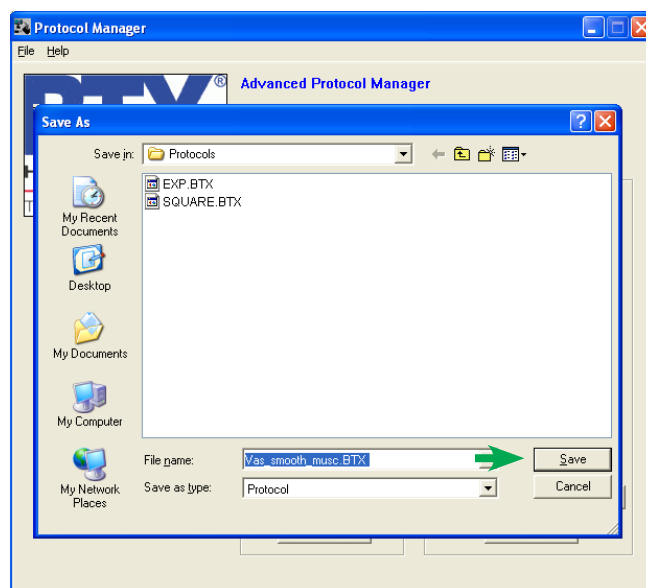


**NOTE:** Hit 'Refresh' to update the list after creating or editing protocols on the Gemini X<sup>2</sup> generator.



3. Select the drive and folder location

4. Click 'Save'



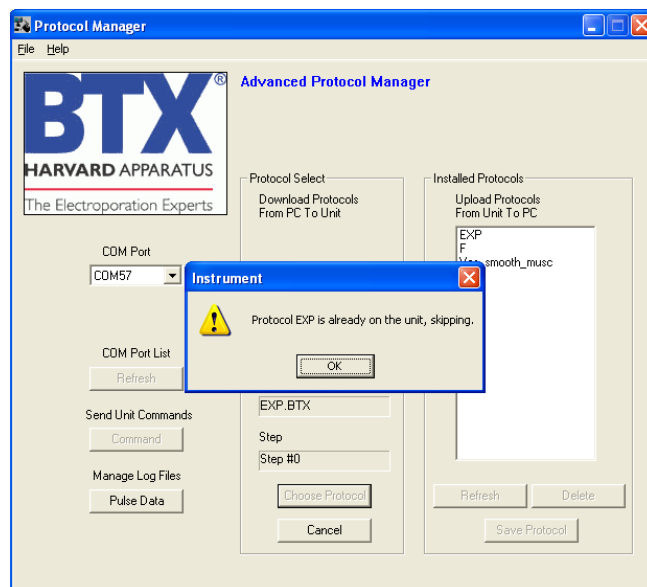
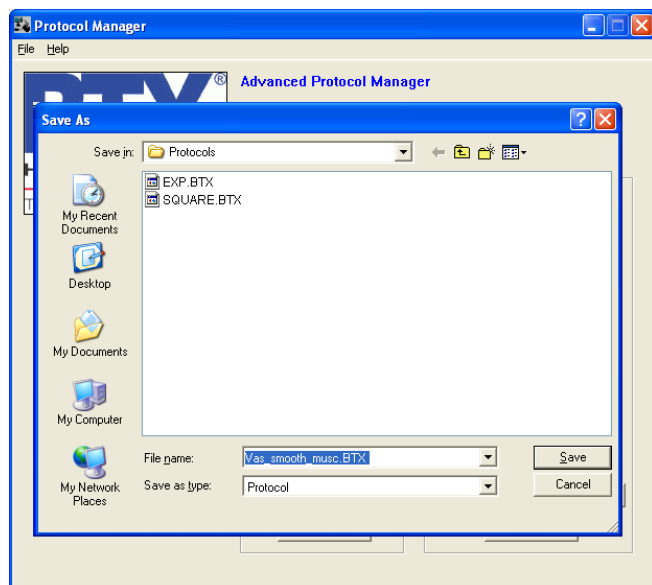
## Protocol Manager

### Download - PC to Generator

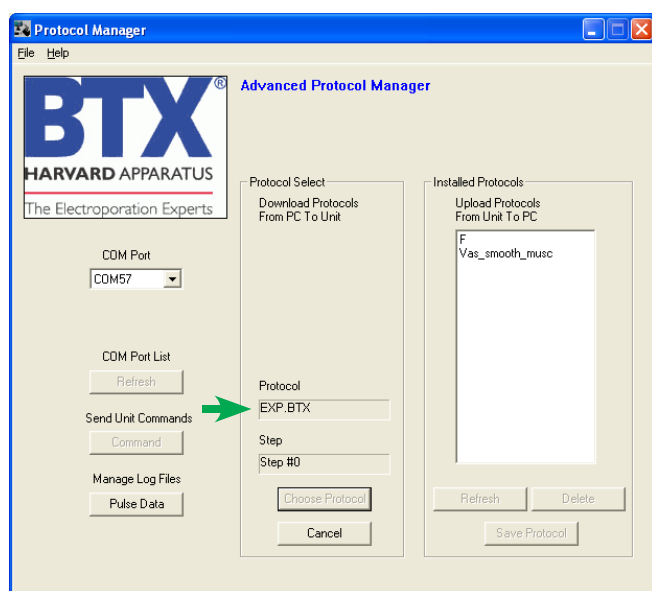
1. Click 'Choose Protocol'
2. Select Protocol(s) to download
3. Click 'Open'



**NOTE:** If a protocol already exists on the Gemini X<sup>2</sup> generator, Protocol Manager will not overwrite the file if you attempt to re-download it.



4. Protocol name being downloaded is shown



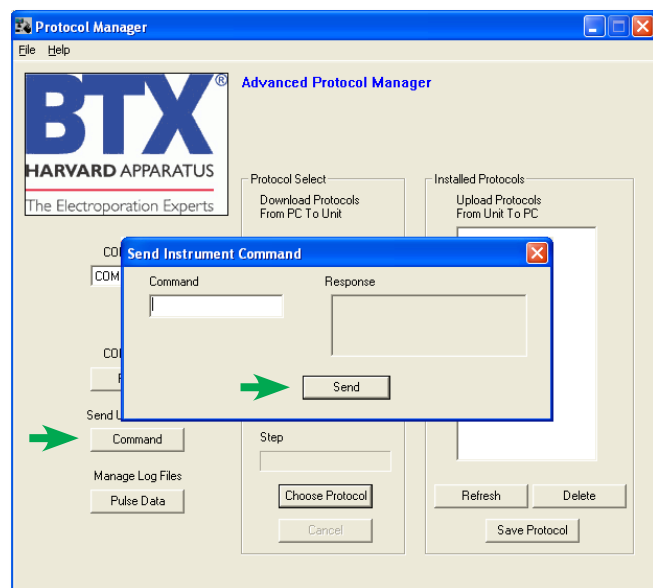
## Protocol Manager

### Sending Commands to Generator

1. Click 'Command'
2. Type desired command
3. Click 'Send'



**NOTE:** If unexpected responses are seen, type 'echo off'.

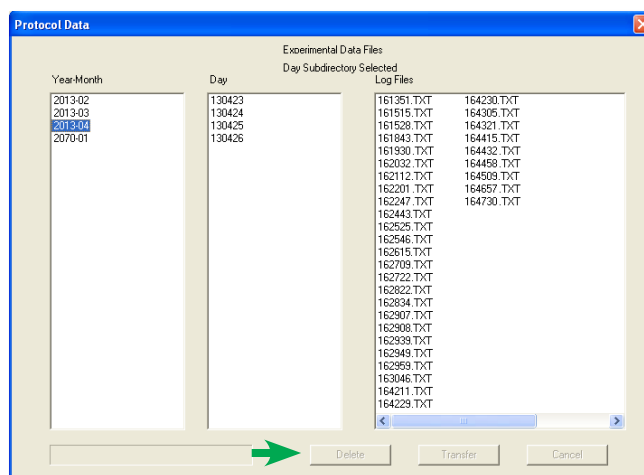


### Upload Log Files

1. On the main screen, click 'Pulse Data'
2. Select your desired files or folders and then click 'Transfer'.



**NOTE:** Files should be backed up periodically. The generator's SD card can store up to approximately 100,000 files.



3. Select the drive or folder location.

### Troubleshooting Protocol Manager

1. Do not upload/download Protocols while generator is running. Program works best while generator is idle on Protocol setup screen.
2. Order of Operations:
  - A. Connect USB.
  - B. Turn generator on.
  - C. Start Protocol Manager program.

## Maintenance

The BTX Gemini Twin Wave Series generators require no special maintenance other than keeping them clean.

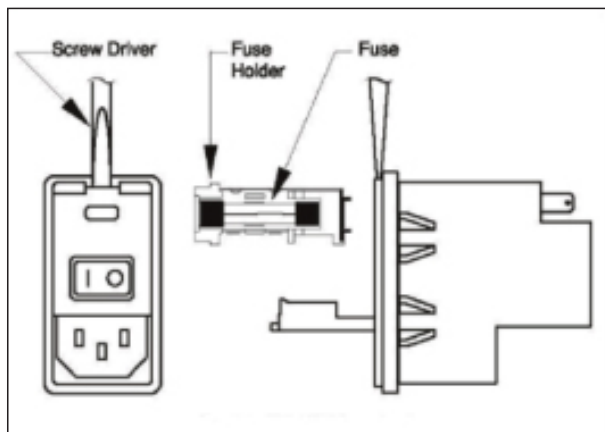
To clean the exterior surfaces, use a lint-free cloth to remove loose dust. Use care to avoid scratching the clear display window. For more efficient cleaning, use a soft cloth dampened (not soaked) with an aqueous solution of 75% isopropyl alcohol, or a mild detergent.

### Fuse Replacement

#### Key Information

1. Make sure the power cord is disconnected from the main supply before servicing the fuse.
2. Use only Type 3AG, 1/4 x 1 1/4 in, 5 amp, 250 volt; Type T (time delay) fuses

Turn off power and remove power cord from power module. Use a straight blade screw-driver to pry open the access door. Remove the fuse holder and then remove the fuses from this holder as shown in figure below. Replace fuses, and then replace the fuse holder.



### Battery Replacement

1. Remove screw and swing cover to the side.
2. Slide battery out from under clip.
3. Install Lithium Coin Battery: 3V, 16mm CR1620 by sliding under clip with positive side facing out



**CAUTION:** Use only same type and rated battery. Observe polarity when installing.





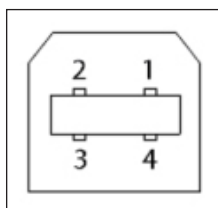
## Upgrading BTX Gemini Series Software

### USB Virtual Commport Driver Installation

When you connect the BTX Gemini X<sup>2</sup> to a computer via USB for the first time, Windows will seek to install a driver for communication. The following section details the installation of the Virtual CommPort Driver supplied with the BTX Gemini X<sup>2</sup>.

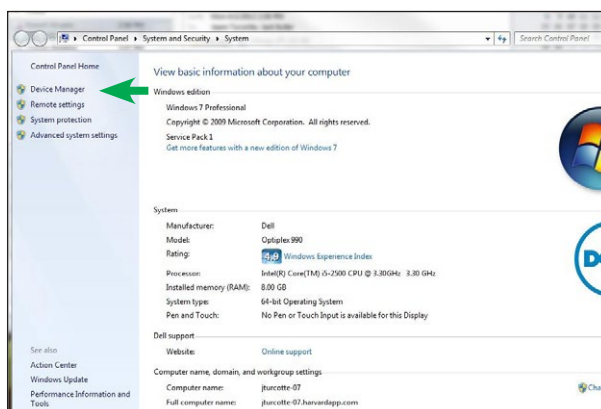
1. Connect the Gemini X<sup>2</sup> generator to the computer via USB. Open the control panel and select **System and Security** and then **System**. Click Device Manager on the left hand menu.

#### Mating Face

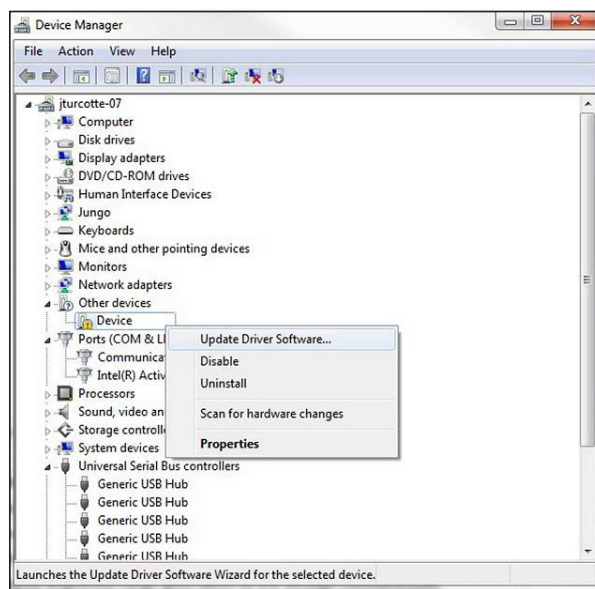


#### Pin # Signal

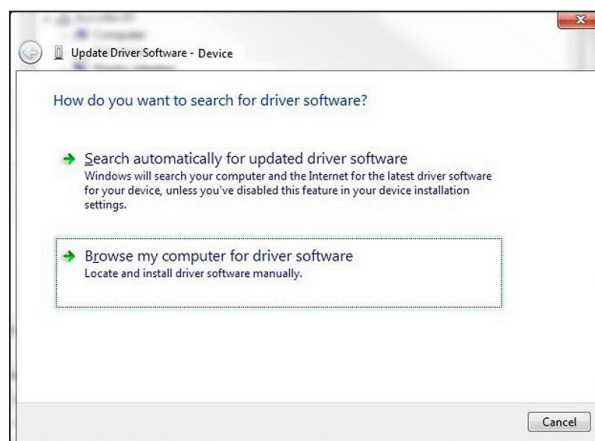
- 1 +5V
- 2 - Data
- 3 + Data
- 4 GND



2. Two Flash update applications will be displayed in the device manager, under **Other Devices**. Right click on either of the flash update applications and select **Update Driver Software**.



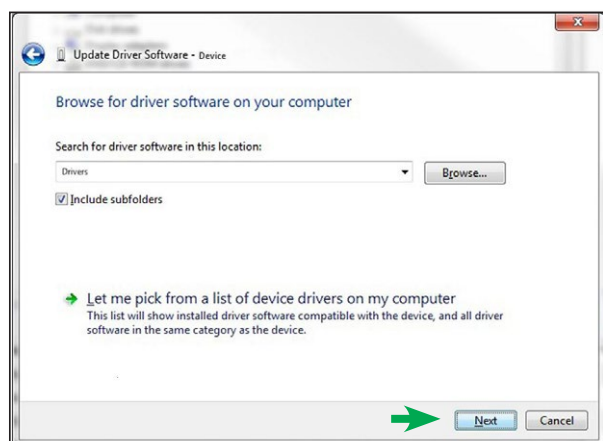
3. Select Browse my computer for driver software



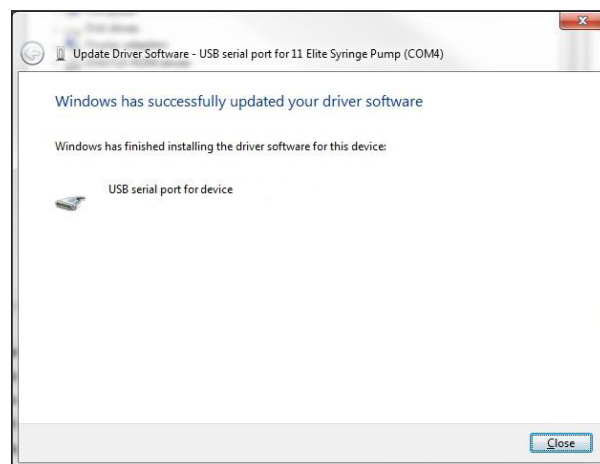
## Upgrading BTX Gemini Series Software

### USB Virtual Comport Driver Installation (continued)

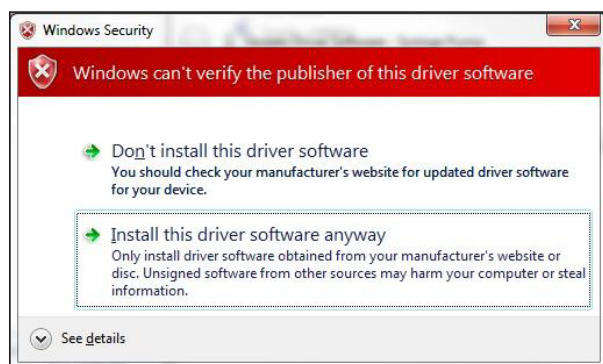
- Click **Browse** and navigate to the generator drivers folder located on the CD supplied with the generator. Select the file BTX USB-CDC.inf. Make sure the **Include Subfolders** box is checked and then click **Next**.



- Click **Close** after the software has been successfully installed.



- If a warning message regarding the driver publisher is displayed, choose **Install this driver software** anyway.



## Upgrading BTX Gemini Series Software

### Device Updater Program



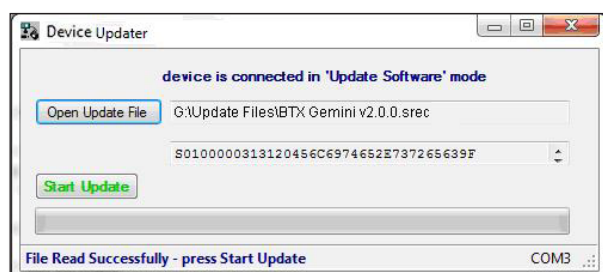
**NOTE:** Prior to upgrading, users will need to install USD drivers as well as the bootloader driver file. Reference 'Virtual Comport Driver Installation'.

1. Upload the latest software versions to your desktop (format is filename.srec). There are two .srec files that both need to be updated: 'BTX Gemini vx.x.x' and a 'BTX Gemini Pwr vx.x.x'.
2. Disconnect all I/O devices and then connect the Gemini X<sup>2</sup> generator to PC using a USB cable.
3. On the Set up screen of the Gemini X<sup>2</sup> generator, press the **Upgrade Software** button twice to enter **Boot Loader Mode**.

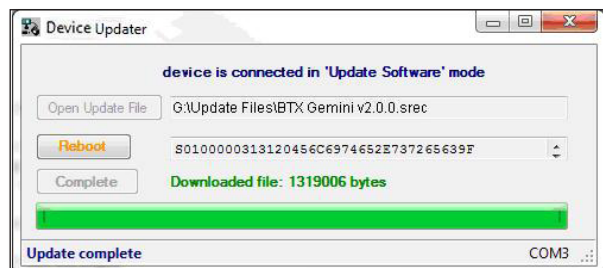


**NOTE:** To exit boot loader mode without upgrading the software, power cycle the generator.

4. From the CD provided with the Gemini X<sup>2</sup> generator, open the **Device Updater Application**.



5. Click Open Update File and browse to the 'BTX Gemini vx.x.x.srec'. file that was previously saved to the desktop.
6. Click **Start Update**.



7. After the update is complete, press Reboot and then power cycle the Gemini X<sup>2</sup> generator from the power switch in the back of the unit.
8. Repeat steps 5 through 7 with the 'BTX Gemini Pwr vx.x.x.srec'.

## Troubleshooting



**NOTE:** It is recommended that you save your protocols periodically by uploading them to a PC.

### Out of Range

A value was entered or encountered in a protocol that was beyond the generator's limits.

SET PROTOCOL VOLTAGE

Voltage 50000\_

HARVARD APPARATUS  
04/03/13 11:06:19 AM

OOR - Volts must be 505 - 3000, and in mults of 5  
PRESS TO CLEAR ERROR

7 8 9  
4 5 6  
1 2 3  
0

CLEAR

### Power Failure Notification

If power is interrupted during operation, an alarm will sound to alert the user to the interruption. When power is restored, an onscreen message is displayed to indicate the interruption.

MAIN MENU

HARVARD APPARATUS  
02/26/13 4:19:11 AM

Preset Protocols

User Protocols

Power failure during last pulse run.  
PRESS TO CLEAR ERROR

Settings

### Low Battery Indication

Time/Date area will turn red when battery is low.

SET DISPLAY BRIGHTNESS

HARVARD APPARATUS  
04/03/13 11:34:54 AM

100% 50%  
90% 40%  
80% 30%  
70% 20%  
60% Set Other Value

CLEAR



## General Optimization Guide for Electroporation

As described, electroporation is the application of controlled direct current (DC) electrical pulses which are applied to living cells and tissues for a short duration of time. The pulse induces a transmembrane potential which causes the reversible breakdown of the cellular membrane. This action results in the permeation or “pore formation” of the cell membrane which allows small molecules (such as dye, oligonucleotides or peptides) and large molecules (such as proteins, DNA and RNA) to be introduced into the cell. During this process the cellular uptake of the molecules continue until the pores close which can take milliseconds to minutes.

Optimization of the electroporation process involves several factors. Choosing the wave form, determining field strength and adjusting pulse length are just a few critical variables. Other parameters which play a crucial role in optimization include cell diameter, plasmid concentrations, temperature and electroporation buffer.

### Wave Forms

Pulse shape generally falls into two categories, square wave or exponential decay wave:

#### Square Wave Pulse

Square wave pulses rise quickly to a set voltage level, maintains this level during the duration of the set pulse length and quickly turns off. Square waves yields higher efficiencies and viabilities in mammalian cells. Square wave EP in **in vivo** and **ex vivo** tissues, embryo's, and plant protoplast applications yield better results in comparison to an exponential decay wave.

#### Exponential Decay Wave Pulse

Exponential decay waves generate an electrical pulse by allowing a capacitor to completely discharge. A pulse is discharged into a sample the voltage rises rapidly to the peak voltage set then declines over time. The powerful exponential decay wave pulse is routinely used for transformation of gram-negative and gram-positive, bacterial, yeast, plant tissues, insect cells and some mammalian cells.

### Field Strength

The field strength is measured as the voltage delivered across an electrode gap and is expressed as kV/cm. Field strength is critical to surpassing the electrical potential of the cell membrane to allow the temporary reversible permeation or “pore formation” to occur in the cell membrane. Three factors should be considered for optimizing field strength:

1. Electrode Gap Size
2. Cell Diameter
3. Temperature

### Cell Types Field Strength Ranges

**Bacteria/Yeast:** 3-24 kV/cm

**Mammalian:** 0.25-3 kV/cm

**Plant:** 3-12 kV/cm

### Electrode Gap Size

The distance between electrodes, or “gap size” is important when optimizing your electroporation experiment. Field strength is calculated using voltage divided by gap size. For example, using a 4mm gap cuvette with 500V would provide a field strength of 1.25kV/cm. If instead of a 4mm gap cuvette, a 2mm gap cuvette was used, the voltage would have to be reduced by half or to 250V in order to maintain the same field strength of 1.25kV/cm. It is possible to derive the voltage needed to accomplish electroporation if the desired field strength and gap size are known. The calculation for this is Field strength (kV) multiplied by gap size (cm) equals voltage. For example, if a user was certain that a 1.25 kV/cm field strength was required in a 1mm gap cuvette the calculation would be: 1.25kV x 0.1cm = 0.125kV or 125V.

Example: A field strength of 1.25 kV/cm

4mm gap cuvette = 500V

2mm gap cuvette = 250V

1mm gap cuvette = 125V

### Cell Diameter

Generally, smaller cell sizes require higher voltages while larger cell diameters require lower voltages for successful cell membrane permeation.

### Temperature

The temperature at which cells are maintained during electroporation effects the efficiency of the electroporation for several reasons. The majority of mammalian cell lines are effectively electroporated at room temperature. Samples which are pulsed at high voltage or exposed to multiple pulses and long pulse durations can cause the sample to heat up. These conditions cause increased cell death and lowers the transfection efficiency. Maintaining the sample at lower temperatures can diminish the heating effects on cell viability and efficiency. 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 exogenous molecules. Yet lower temperatures on other cell lines can be damaging and cause high cell mortality. This effect is specific to each cell line and should be considered during optimization studies. The standard pulse voltage used for cells at room temperature will need to be approximately doubled for electroporation at 4°C in order to effectively permeate the cell membrane.



## General Optimization Guide for Electroporation

### Pulse Length

The pulse length is the duration of time the sample is exposed to the pulse. This is measured as time in micro to milliseconds ranges. Adjusting this parameter is dependent on the pulse wave form. The pulse length in a square wave system can be inputted directly. The pulse length in an exponential decay wave system is called the "time constant" which is characterized by the rate at which the pulsed energy (e) or voltage is decayed to 1/3 the original set voltage. This time constant is modified by adjusting the resistance and capacitance (RC) values in an exponential decay wave form. Time constant calculation  $T=RC$ , where T is time and R is resistance and C is capacitance.

The pulse length works indirectly with the field strength to increase pore formation and therefore the uptake of target molecules. Generally, during optimization of parameters an increase in voltage should be followed by an incremental decrease in pulse length. When decreasing the voltage, the reverse is true. Pulse length is a key variable that works hand in hand with voltage and needs to be considered when optimizing electrical parameters to maximize the results for a given cell type.

### Number of Pulses

Electroporation is typically carried out as a single pulse for most cell types. However, other cell lines may require multiple pulses to achieve maximum transfection efficiencies. Usually lower voltages are used when applying multiple pulses in order to gradually permeate the cell membranes. This allows the transfer of molecules while avoiding damage to delicate or whole tissue samples. This method of multiple pulsing is critical for maximum gene delivery without causing tissue damage to *in vivo*, *in utero* and explant tissue environments. The use of multiple pulse will require the optimization of key electrical parameters including voltage and pulse length. Typically, for *in vivo* applications the use of lower voltages between 10-100 volts with pulse lengths ranging 30-50msec provides efficient transfection. The optimal voltage, pulse length and number of pulses will vary depending on the cell type and molecule (DNA or RNA) transfected.

### Electroporation Buffer

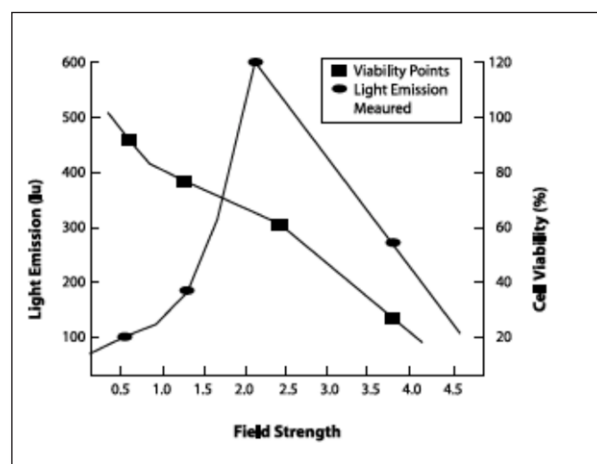
The buffers used for electroporation can vary depending on the cell type. Many applications use highly conductive buffers such as PBS (Phosphate Buffered Saline <30 ohms) and HBSS (Hepes Buffer <30 ohms) or standard culture media which may contain serum. Other recommended buffers are hypoosmolar buffers in which cells absorb water shortly before pulse. This swelling of the cells results in lowering the optimal permeation voltage while ensuring the membrane is more easily permeable for many cells but can be damaging to others. Prokaryotic cells such as bacteria require the use of high resistance buffers (>3000 ohms) for this reason proper preparation and washing of the cells is essential to remove excess salt ions to reduce the chance of arcing. Ionic strength of an electroporation buffer has a direct affect on the

resistance of the sample which in turn will affect the pulse length or time constant of the pulse. The volume of liquid in a cuvette has significant effect on sample resistance for ionic solutions, the resistance of the sample is inversely proportional to the volume of solution and pH. As the volumes are increased resistance decreases which increases the chance of arcing, while lowering the volume will increase the resistance and decrease the arc potential.

Harvard Apparatus BTX now offers BTXpress High Performance Electroporation Solution, a low conductance buffer that achieves higher transfection efficiencies with minimal cell toxicity. The BTXpress buffer is a single buffer developed to facilitate high efficiency gene delivery into mammalian cells.

### DNA/RNA Concentrations

Electroporation is typically thought of as a nucleic acid (DNA, mRNA, siRNA and miRNA) transfer method into prokaryotic and eukaryotic cells. Electroporation is not limited to just nucleic acid delivery, it can introduce proteins, antibodies, small molecules and fluorescent dyes. The standard range of DNA used for transfections is 5-20g/ml for most cell types; however in some instances increasing the DNA concentration as high as 50g/ml improves transfection efficiency without changing other parameters. Determining the optimal DNA concentration through a DNA titration can be beneficial. The size of a molecule will have an effect on the electrical parameters used to transfect the cell. Smaller molecules (siRNA or miRNA) may need higher voltage with microsecond pulse lengths and larger molecules (DNA) may need lower voltages with longer pulse lengths. Buffers such as EDTA or Tris can drastically reduce the transfection efficiency. Therefore, we recommend resuspending DNA in distilled water. Finally, electroporating ligation mixtures into E.coli can cause arcing and reduced transformations. Diluting the ligation mixture a minimum of 1:5 with diH<sub>2</sub>O, dialysis, or ethanol precipitation can significantly improve transformation efficiencies and reduce the potential for arcing.







## Applications

### Mammalian Cell Transfection

Electroporation is a highly flexible technique used to genetically modify mammalian cells. Whether you are studying up or down regulation of genes, specific protein expression. This method is non-toxic and requires no expensive reagents to successfully transfect your cells. Primary cells, stem cells or established cell lines can be electroporated with yield high transfection efficiencies and great cell survival rates.

### In Vivo, In Utero, In Ovo

Square waves system allow researchers to set the pulse lengths and number of pulses which is critical to ensure viable cells and tissues while still maintaining efficient transfection both *in vivo* and *ex vivo*. Electroporation mediated gene and drug delivery has been shown to substantially increase intracellular uptake and expression of DNA, siRNA and miRNA in muscle, skin, liver, kidney, testis, retina, tumors, etc. In vivo electroporation has successfully been used in embryo applications, *in utero* and *in ovo* applications in addition to transfection of Zebra fish.

### Bacteria and Yeast Transformation

Electroporation has long been recognized as the most efficient means of transforming both gram negative and gram positive bacteria and yeast. Gramnegative bacteria such as coli or Helicobacter pylori are generally easier to transform than grampositive bacteria (e.g. Streptococcus pneumoniae) due to their cell wall composition. Transformation efficiencies of  $1 \times 10^{10}$  transformants/ $\mu$ g DNA are commonly seen for gram-negative bacteria, while for gram-positive bacteria, generally  $1 \times 10^6$  transformants/ $\mu$ g DNA are achievable.

### Plant and Insect Transfection

Electroporation of plant tissue can be used to generate transgenic crops that are useful in agricultural/horticultural applications.

Insect models are also widely used throughout the scientific community to study development and gene regulation and function. The ability to introduce genes or molecules is essential to researchers working with either of these two species. This is why researchers consistently turn to Harvard Apparatus BTX for all of their electroporation needs.

### References

- Jonathan M. Dermott, J. M. Gooya, B. Asefa, S. R. Weiler, M. Smith, J. R. Keller. Inhibition of Growth by p205: A nuclear protein and putative tumor suppressor expressed during Myeloid Cell differentiation. *Stem Cells* 22:832-848. 2004
- Jonathan M. Quinlan, Wei-Yuan Yu, Mark A. Hornsey, David Tosh and Jonathan MWSlack. In vitro culture of embryonic mouse intestinal epithelium: cell differentiation and introduction of reporter genes. *BMC Developmental Biology* 6:24. 2006
- Yangbing Zhao, Zhili Zheng, Cyrille J. Cohen, Luca Gattinoni, Douglas C. Palmer, Nicholas P. Restifo, Steven A. Rosenberg, and Richard A. Morgan. High-efficiency transfection of primary human and mouse T lymphocytes using RNA electroporation. *Molecular Therapy* (2006) 13, 151-159

William J. Buchser, Jose R. Pardinas, Yan Shi, John L. Bixby, and Vance P. Lemmon. 96-Well electroporation method for transfection of mammalian central neurons. *BioTechniques* Vol. 41, no. 5. 2006

K. Regha, Ajit K. Satapathy and Malay K. Ray. RecD plays an essential function during growth at low temperature in the Antarctic Bacterium *Pseudomonas syringae* Lz4W. *Genetics* 170: 1473-1484. August 2005

Victor B. Busov, R. Meilan, D.W. Pearce, C. Ma, S. B. Rood, and S. H. Strauss. Activation Tagging of a dominant gibberellin catabolism gene (GA 2-oxidase) from poplar that regulates tree stature. *Plant Physiology*, Vol. 132, pp. 1283-1291. July 2003

Jun Ishikawa, Kazuhiro Chiba, Haruyo Kurita, and Hiroyuki Satoh. Contribution of rpoB2 RNA Polymerase, Subunit Gene to Rifampin Resistance in *Nocardia* Species. *Antimicrobial Agents Chemotherapy*, 50(4):1342-1346. April 2006

Bindu Garg, Romesh C. Dogra, and Parveen K. Sharma. High-Efficiency Transformation of *Rhizobium leguminosarum* by Electroporation. *Applied Environmental Microbiology*. 65(6):2802-2804. June 1999

### General References

#### In vitro Electroporation

Kim, T. et. al., Mesoporous Silica-Coated Hollow Manganese Oxide Nanoparticles as Positive T1 Contrast Agents for Labeling and MRI Tracking of Adipose-Derived Mesenchymal Stem Cells. *J. Am. Chem. Soc.*, 133, 2955-2961, 2011

Kataoka, N. et. al., Development of butanol-tolerant *Bacillus subtilis* strain GRSV2-B1 as a potential bioproduction, *AMB Express*, 1:10, 2011

Hutson, T.H. et. al., Optimization of a 96 well electroporation assay for post natal rat CNS neurons suitable for cost-effective medium-throughput screening of genes that promote neurite outgrowth. *Frontiers in Molecular Neuroscience*; 4(55): December 2011

Djouad, F. et. al., Activin A expression regulates multipotency of mesenchymal progenitor cells. *Stem Cell Res & Therapy*, 1(11), 2010

Sankaranarayanan, K. et. al., Electro-Molecular Therapy using Adult Mesenchymal Stem Cells. *Proc. ESA Annual Meeting on Electrostatics*, 13, 2010

Blackmore, M. et. al., High content screening of cortical neurons identifies novel regulators of axon growth. *Molecular and Cellular Neuroscience*, 44, 43-54, 2010

Yao, S. et. al., Improvement of electroporation to deliver plasmid DNA into dental follicle cells. *Biotechnol J.* October; 4(10): 1488-1496. 2009

Rinaldi, G., Development of Functional Genomic Tools in Trematodes: RNA Interference and Luciferase Reporter Gene Activity in *Fasciola hepatica*. *PLoS One*, 2(7); e260, July 2008

Nguyen, K. et al., T Cell Costimulation via the Integrin VLA-4 Inhibits the Actin-Dependent Centralization of Signaling Microclusters Containing the Adaptor SLP-76. *Immunity*, 28, 810-821, June 2008

Yang, C. et al., Dimeric heat shock protein 40 binds radial spokes for generating coupled power strokes and recovery strokes of 9 + 2 flagella. *The Journal of Cell Biology*, 180(2), pp. 403-415, January 28, 2008



## Applications

### General References (continued)

#### *In Vitro* Electroporation (continued)

Zhao, Y. et al., High-Efficiency Transfection of Primary Human and Mouse T Lymphocytes Using RNA Electroporation. *Molecular Therapy*, Vol. 13, No. 1, January 2006

Heaney, J.D. et. al. Tissue-specific expression of a BAC transgene targeted to the *Hprt* locus in mouse embryonic stem cells. *Genomics*, 2004

Raoul, C. et al., Motoneuron Death Triggered by a Specific Pathway Downstream of Fas: Potentiation by ALS-Linked SOD1 Mutations. *Neuron*, Vol. 35, 1067-1083, September 12, 2002

Dawson, K. et al., Insulin-Regulated Trafficking of Dual-Labeled Glucose Transporter 4 in Primary Rat Adipose Cells, *Biochemical and Biophysical Research Communications*. 287, pp. 445-454, 2001

#### *In Vivo* Electroporation

Johansson, D. et. al., Intradermal Electroporation of Naked Replicon RNA Elicits Strong Immune Responses. *PLoS ONE*, 7(1): e29732, 2012

Daftarian, P. et. al., In vivo Electroporation and Non-protein Based Screening Assays to Identify Antibodies Against Native Protein Conformations. *Hybridoma*, 30(5); 2011

Hallengard, D. et. al., Comparison of plasmid vaccine immunization schedules using intradermal in vivo electroporation. *Clinical Vaccine Immunology*, 2011

Bolhassani, A. et. al., Improvement of different vaccine delivery systems for cancer therapy. *Molecular Cancer*, 10(3), 2011

Li, W. et. al., The Effects of Irreversible Electroporation (IRE) on Nerves. *PLoS ONE*, 6(4), 2011

Lladser, A. et. al., Intradermal DNA electroporation induces survivin-specific CTLs, suppresses angiogenesis and confers protection against mouse melanoma. *Cancer Immunol Immunother*, 59; 81-92, 2010

Shi, W. et al., Generation of sp3111 transgenic RNAi mice via permanent integration of small hairpin RNAs in repopulating spermatogonial cells in vivo. *Acta Biochim Biophys Sci*, 42(2): p 116, 2010

Haller, BK. et. al., Therapeutic efficacy of a DNA vaccine targeting the endothelial tip cell antigen delta-like ligand 4 in mammary carcinoma. *Oncogene*, 29, 4276-4286, 2010

Guo, Y. et. al., Irreversible Electroporation Therapy in the Liver: Longitudinal Efficacy Studies in a Rat Model of Hepatocellular Carcinoma. *Cancer Research*; 70(4) February 15, 2010

Roos, A. K. ,Skin Electroporation: Effects on Transgene Expression, DNA Persistence and Local Tissue Environment. *PLoS ONE*, 4(9) e7226, 2009

Brave, A. et. al., Late administration of plasmid DNA by intradermal electroporation efficiently boosts DNA-primed T and B cell responses to carcinoembryonic antigen. *Vaccine*, 27, 3692-3696, 2009

Roos, A. K. et. al., Optimization of Skin Electroporation in Mice to Increase Tolerability of DNA Vaccine Delivery to Patients. *Molecular Therapy*, 17(9), 1637-1642, Sep 2009

Danner, S. et. al., Seminiferous tubule transfection in vitro to define post-meiotic gene regulation. *Reproductive Biology and Endocrinology*, 7(67), 2009

Benton, C. et al., Modest PGC-1<sub>α</sub> Overexpression in Muscle in Vivo Is Sufficient to Increase Insulin Sensitivity and Palmitate Oxidation in Subsarcolemmal, Not Intermembranous, Mitochondria\*. *The Journal of Biological Chemistry*, 283(7); pp. 4228-4240, February 15, 2008

Chesler, A. T., Selective Gene Expression by Postnatal Electroporation during Olfactory Interneuron Neurogenesis. *PLoS ONE*, 3(1): e1517, 2008

Rao, N. M. et al., Electroporation of Adult Zebrafish. S. Li (ed.), *Electroporation Protocols: Preclinical and Clinical Gene Medicine*. *Methods in Molecular Biology*, Vol. 423. p 289, 2008

Johnson, C. J. et al., Technical Brief: Subretinal injection and electroporation into adult mouse eyes. *Molecular Vision*, 14:2211-2226, 2008

Heller, L. et. al., Comparison of electrically mediated and liposome-complexed plasmid DNA delivery to the skin. *Genetic Vaccines and Therapy*, 6(16), 2008

Roos, A.K., et. al., Enhancement of Cellular Immune Response to a Prostate Cancer DNA Vaccine by Intradermal Electroporation. *Molecular Therapy*, 13(2), February 2006

Kong, X. C. et al., Inhibition of synapse assembly in mammalian muscle in vivo by RNA interference. *EMBO Rep*, 5(2): 183-188, January 2004

Pringle, I. A. et al., Duration of reporter gene expression from naked pDNA in the mouse lung following direct electroporation and development of wire electrodes for sheep lung electroporation studies. *Molecular Therapy*, 9, S56-S56, 2004

Mikata, K. et al., Inhibition of Growth of Human Prostate Cancer Xenograft by Transfection of p53 Gene: Gene Transfer by Electroporation. *Molecular Cancer Therapeutics*, Vol. 1, 247-252, February 2002

Pekarik, V. et al., Screening for gene function in chicken embryo using RNAi and electroporation. *Nature Biotechnology*, 21: 93-96, December 2002

Dujardin, N. et. al., In vivo assessment of skin electroporation using square wave pulses. *J Controlled Release*, 79, 219-227; 2002

Drabick, J.J. et. al., Cutaneous Transfection and Immune Responses to Intradermal Nucleic Acid Vaccination Are Significantly Enhanced by in Vivo Electroporation. *Molecular Therapy*, 3(2), Feb 2001

#### *In Utero* Electroporation

Maiorano, N. A., et al., Promotion of embryonic cortico-cerebral neurogenesis by miR-124. *Neural Development*, 4:40, 2009

#### *Ex Vivo* Electroporation

Deora, A.A. et. al., Efficient Electroporation of DNA and Protein into Confluent and Differentiated Epithelial Cells in Culture. *Traffic*, 8: 1304-1312, 2007

Thomas J-L. et. al., Electroporation, an alternative to biolistics for transfection of *Bombyx mori* embryos and larval tissues. *Journal of Insect Science*, 3:17, 2003

Dimitrov, D.S., and Sowers, A.E., (1990) Membrane electroporation - fast molecular exchange by electroosmosis. *Biochimica et Biophysica Acta* 1022: 381-392.



## Applications

### General References (continued)

#### Ex Vivo Electroporation (continued)

Deora, A.A. et. al., *Efficient Electroporation of DNA and Protein into Confluent and Differentiated Epithelial Cells in Culture*. *Traffic*, 8: 1304-1312, 2007

Thomas J-L. et. al., *Electroporation, an alternative to biolistics for transfection of Bombyx mori embryos and larval tissues*. *Journal of Insect Science*, 3:17, 2003

Dimitrov, D.S., and Sowers, A.E., (1990) *Membrane electroporation - fast molecular exchange by electroosmosis*. *Biochimica et Biophysica Acta* 1022: 381-392.

Sukharev SI, Klenchin VA, Serov SM, Chernomordik LV and Chizmadzhev YA, (1992) *Electroporation, and electrophoretic DNA transfer into cells: The effect of DNA interaction with electropores*, 1992, *Biophys J.* 63: 1320-1327.

Nickoloff, Jac A., ed. (1995) *Plant Cell Electroporation and Electrofusion Protocols, Methods in Molecular Biology, Volume 55*. (Humana Press, Totowa, New Jersey).

E. A. Disalvo and S.A. Simon, eds. (1995) *Permeability and Stability of Lipid Bilayers* (CRC Press, Boca Raton), p 105-121.

Chang, D.C., Chassy, B.M., Saunders, J.A. and Sowers, A.E., eds. (1992) *Guide to Electroporation and Electrofusion*, (Academic press, San Diego), 581 pp.

Neuman, E., Sowers, A.E., and Jordan, C.A., eds. (1989) *Electroporation and Electrofusion in Cell Biology*, (Plenum Press, New York) 581 pp.

Bartoletti, D. C., Harrison, G. I., & Weaver, J. C. (1989). *The number of molecules taken up by electroporated cells: quantitative determination*. *FEBS Lett.*, 256, 4-10.

Djuzenova, C. S., Zimmermann, U., Frank, H., Sukhorukov, V. L., Richter, E., & Fuhr, G. (1996). *Effect of medium conductivity and composition on the uptake of propidium iodide into electroporeabilized myeloma cells*. *Biochim.Biophys.Acta*, 1284, 143-152.

Klenchin VA, Sukharev SM, Chernomordik LV, Chizmadzhev YA, *Electrically induced DNA uptake by cells is a fast process involving DNA electrophoresis*, 1991, *Biophys J.* 60:804-811 Neumann, E., Kakorin, S., & Toensing, K. (1999). *Fundamentals of electroporative delivery of drugs and genes*. *Bioelectrochem.Bioenerg.*, 48, 3-16.

Neuman, E., Toensing, K., Kakorin, S., Budde, P., & Frey, J. (1998). *Mechanism of electroporative dye uptake by mouse B cells*. *Biophys.J.*, 74, 98-108. Sukharev, S. I., Klenchin, V. A., Serov, S. M., Chernomordik, L. V., & Chizmadzhev, Y. (1992). *Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores*. *Biophys.J.*, 63, 1320-1327.

Wolf, H., Rols, M. P., Boldt, E., Neumann, E., & Teissie, J. (1994). *Control by pulse parameters of electric field-mediated gene transfer in mammalian cells*. *Biophys.J.*, 66, 524-531.

Zerbib, D., Amalric, F., & Teissie, J. (1985). *Electric field mediated transformation: isolation and characterization of a TK+ subclone*. *Biochem.Biophys.Res.Comm.*, 129, 611-618.



## Electroporation Buffers

There are many kinds of buffers and medium used for electroporating cells. Typically, we recommend using medias without serum or antibiotics.

The following is a list of the most commonly used buffers/medium:

**BTXpress** – is a single buffer solution, developed to quickly and efficiently deliver genes into mammalian cells that were previously considered “hard to transfect” by chemical and other non-viral methods. This solution, in combination with BTX electroporators, provides researchers with the versatility needed for success across a broad range of cell types while maintaining critical cell viability. Transfection using this high performance electroporation solution is equally effective in delivering DNA as well as siRNA into mammalian cells.

**PBS** – is a buffer solution commonly used in biological research. It is a water-based salt solution containing sodium chloride, sodium phosphate, and, in some formulations, potassium chloride and potassium phosphate. The buffer’s phosphate groups help to maintain a constant pH. The osmolarity and ion concentrations of the solution usually match those of the human body (isotonic).

**HEPES** – is widely used in cell culture, largely because it is better at maintaining physiological pH despite changes in carbon dioxide concentration (produced by cellular respiration) when compared to bicarbonate buffers, which are also commonly used in cell culture.

**RPMI** – is a form of medium used in cell culture and tissue culture. It has traditionally been used for growth of Human lymphoid cells. This medium contains a great deal of phosphate and is formulated for use in a 5% carbon dioxide atmosphere.

**Opti-MEM** – is an improved Minimal Essential Medium (MEM) that allows for a reduction of Fetal Bovine Serum supplementation by at least 50% with no change to growth rate or morphology. Opti-MEMt can be used with a variety of suspension and adherent mammalian cells, including Sp2, AE-1, CHO, BHK-21, HEK, and primary fibroblasts.

**MEM Eagle** – is suitable for a diverse spectrum of mammalian cell types. Various formulations available with either Hank’s or Earle’s salts.

**DMEM** – MEM is used in a wide range of mammalian cell culture applications. The high glucose version is well suited to high density suspension culture. The low glucose formula is used for adherent dependent cells.

**CytoMix** – is a composition of cytokines for the highly efficient and reproducible expansion of human multipotent mesenchymal stromal cells (MSCs).

**Water & 10% glycerol** – Typically used for bacteria

**Cytoporation Media T** – is a buffer designed for larger volume cell electroporation as it incorporates a low conductivity of 0.08 S/cm to reduce heating of solution during electroporation.

**Cytoporation Media T4** – is a buffer designed for larger volume cell electroporation as it incorporates a low conductivity of 3.45 mS/cm to reduce heating of solution during electroporation.

## Experiment 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, 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.

### Low Viability

Verify physical, biological, chemical parameters. Is 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 .

### Voltage Drop

A drop in output voltage accompanies pulse delivery into highly conductive samples (for example, PBS). Thus the displayed voltage may in these situations be less than that expected, given 5% full scale accuracy and the monitoring accuracy of 5%.





## Ordering Information

Order No.	Description	UOM
<b>Electroporation Systems</b>		
<b>45-2001</b>	BTX Gemini SC <sup>2</sup> Generator only	1 ea
<b>45-2002</b>	BTX Gemini SC <sup>2</sup> Electroporation System. Includes Gemini SC <sup>2</sup> Generator; Cuvettes - 610, 620, 640 pack of 30 (10 ea), Safety Dome 1, and 660 Cuvette Rack	1 ea
<b>45-2006</b>	BTX Gemini X <sup>2</sup> Generator only	1 ea
<b>45-2007</b>	BTX Gemini X <sup>2</sup> Electroporation System. Includes Gemini X <sup>2</sup> Generator; Cuvettes - 610, 620, 640 pack of 30 (10 ea), Safety Dome 2, and 660 Cuvette Rack	1 ea
<b>45-2008</b>	BTX Gemini X <sup>2</sup> HT Electroporation System. Includes Gemini X <sup>2</sup> Generator; Cuvettes - 610, 620, 640 pack of 30 (10 ea), Safety Dome 2, HT 200 Plate Handler, 1 x 2 mm gap HT Plate, 1 x 4 mm HT Plate and 660 Cuvette Rack	1 ea
<b>Cuvettes</b>		
<b>45-0124</b>	Cuvette Plus, 1 mm gap, 90 µl, pk/50, Gray	50 pk
<b>45-0125</b>	Cuvette Plus, 2 mm gap, 400 µl, pk/50, Blue	50 pk
<b>45-0126</b>	Cuvette Plus, 4 mm gap, 800 µl, pk/50, Yellow	50 pk
<b>45-0140</b>	Bulk Cuvette, 1 mm, 24 pk/100, Gray Case	2400 ea
<b>45-0141</b>	Bulk Cuvette, 2 mm gap, 24 pk/100, Blue Case	2400 ea
<b>45-0142</b>	Bulk Cuvette, 4 mm gap, 24 pk/100, Yellow Case	2400 ea
<b>HT Plates (Gemini X<sup>2</sup> use only)</b>		
<b>45-0462</b>	25 Well Plate, 4 mm gap, 250 µl	1 ea
<b>45-0463</b>	25 Well Plate, 4 mm gap, 250 µl, pk/6	6 pk
<b>45-0466</b>	25 Well Plate, 2 mm gap, 125 µl	1 ea
<b>45-0467</b>	25 Well Plate, 2 mm gap, 125 µl, pk/6	6 pk
<b>45-0450</b>	96 Well Plate, 2 mm gap, 125 µl	1 ea
<b>45-0452</b>	96 Well Plate, 4 mm gap, 250 µl	1 ea
<b>Reagents</b>		
<b>45-0802</b>	BTXpress, 5 ml	1 ea
<b>45-0803</b>	BTXpress, 5 ml with 2 mm gap, Cuvettes pk/50	1 ea
<b>45-0804</b>	BTXpress, 5 ml with 4 mm gap, Cuvettes pk/20	1 ea
<b>45-0805</b>	BTXpress, 10 ml	1 ea
<b>45-0806</b>	BTXpress, 10 ml with 2 mm gap, Cuvettes pk/50	1 ea
<b>45-0807</b>	BTXpress, 10 ml with 4 mm gap, Cuvettes pk/40	1 ea
<b>47-0002</b>	Cytoporation Media T 500 ml	1 ea
<b>47-0003</b>	Cytoporation Media T4 500 ml	1 ea

Order No.	Description	UOM
<b>Accessories</b>		
<b>45-0400</b>	HT 100 Plate Handler Manual	1 ea
<b>45-0401</b>	HT 200 Plate Handler Auto	1 ea
<b>45-2020</b>	BTX Safety Dome for SC <sup>2</sup>	1 ea
<b>45-2021</b>	BTX Safety Dome for X <sup>2</sup>	1 ea
<b>45-2030</b>	BTX Gemini X <sup>2</sup> Footswitch	1 ea
<b>45-0208</b>	Cuvette Rack	1 ea
<b>45-0465</b>	HT 25 Well Adapter Plate	1 ea
<b>45-0468</b>	Plate Handler Pins, pk/25	25 ea
<b>45-0469</b>	Plate Handler Pins, pk/100	100 ea
<b>5012-017</b>	Pliers for Plate Handler Removing Pins	1 ea
<b>4500-012</b>	25 Well Plate Seal	2 ea
<b>4500-015</b>	96 Well Plate Seal	2 ea
<b>Cables</b>		
<b>45-0216</b>	Connection Cable Cable, 10 ft, Banana to Micrograbber	1 ea
<b>45-0204</b>	Tweezertrode Cable / Single Adaptor Cable for Tissue Slice Electrode Positive	1 ea
<b>45-0503</b>	Mini Micro Grabber Adapter Cables for Tissue Slice Chamber / L Shaped Needle Electrodes	1 ea
<b>45-0087</b>	Micrograbber to Banana Adapter Set 45-0217	1 pr
<b>45-2031</b>	BTX USB Cable, 2 m	1 ea
<b>45-2032</b>	BTX USB Cable, 5 m	1 ea
<b>45-0217</b>	Electrode Cable for Flat Electrode, 10 ft BTB	1 ea
<b>45-0088</b>	Banana Splice F/F Adapter Set, 45-0216/45-0217	1 pr
<b>45-0090</b>	Adapter Set Banana to Pin Tip	1 ea
<b>45-0089</b>	Banana to Square Post Adapter Set for 45-0217	1 pr



## Ordering Information

Order No.	Description	UOM
<b>45-0101</b>	Caliper Electrode, 1.0 x 1.0 cm Kit	1 Ea
<b>45-0102</b>	Caliper Electrode, 2.0 x 2.0 cm, 1.5 x 1.5 cm Kit	1 Ea
<b>45-0103</b>	Microslide 450, 0.5 mm gap, 20 µl, pk/10	10 Pk
<b>45-0104</b>	Microslide 450-I, 1 mm gap, 40 µl, pk/10	10 Pk
<b>45-0105</b>	Microslide 453, 3.2 mm gap, 650 µl	1 Ea
<b>45-0106</b>	Microslide 453-10, 10 mm gap, 2.0 ml	1 Ea
<b>45-0107</b>	Meander Fusion Chamber, 0.2 mm gap, pk/4	4 Pk
<b>45-0108</b>	Flat Electrode/Divergent Field, 1 mm (needs 45-0217)	1 Ea
<b>45-0217</b>	Electrode Cable for Flat Electrode, 10 ft BTB	1 Ea
<b>45-0109</b>	Flatpack Chambers, 1.83 mm gap, pk/50	50 Pk
<b>45-0110</b>	Flatpack Chambers, 0.56 mm gap, pk/50	50 Pk
<b>45-0113</b>	Genetrodes Straight, 5 mm, Gold Tip	1 Ea
<b>45-0160</b>	Genetrodes Straight, 5 mm, Gold Tip Kit	1 Ea
<b>45-0114</b>	Genetrodes Straight, 10 mm, Gold Tip	1 Ea
<b>45-0161</b>	Genetrodes Straight, 10 mm, Gold Tip Kit	1 Ea
<b>45-0115</b>	Genetrodes L-Shape, 5 mm, Gold Tip	1 Ea
<b>45-0162</b>	Genetrodes L-Shape, 5 mm, Gold Tip Kit	1 Ea
<b>45-0116</b>	Genetrodes L-Shape, 3 mm, Gold Tip	1 Ea
<b>45-0163</b>	Genetrodes L-Shape, 3 mm, Gold Tip Kit	1 Ea
<b>45-0117</b>	Genetrodes L-Shape, 1 mm, Gold Tip	1 Ea
<b>45-0164</b>	Genetrodes L-Shape, 1 mm, Gold Tip Kit	1 Ea
<b>45-0203</b>	Genetrodes/Genepaddle Holder with Shaft	1 Ea
<b>45-0216</b>	Genetrodes/Genepaddle Cable, 10 ft, BTMG	1 Ea
<b>45-0122</b>	Genepaddles, 3 x 5 mm	1 Ea
<b>45-0169</b>	Genepaddle, 3 x 5 mm Kit	1 Ea
<b>45-0123</b>	Genepaddles, 5 x 7 mm	1 Ea
<b>45-0170</b>	Genepaddle, 5 x 7 mm Kit	1 Ea
<b>45-0167</b>	2-Needle Array, 10 mm Kit	1 Ea
<b>45-0205</b>	2-Needle Array Handle, 10 mm (needs 45-0120)	1 Ea
<b>45-0120</b>	2-Needle Array, 10 mm, pk/6 (needs 45-0205)	1 Ea
<b>45-0168</b>	2-Needle Array, 5 mm Kit	1 Ea
<b>45-0206</b>	2-Needle Array Handle, 5 mm (needs 45-0121)	1 Ea
<b>45-0121</b>	2-Needle Array, 5 mm pk/6 (needs 45-0206)	1 Ea
<b>45-0510</b>	Needle L-Shaped Pt Electrode, 3 mm Kit	1 Ea
<b>45-0509</b>	Needle L-Shaped Pt Electrode, 3 mm (needs 45-0508)	1 Ea
<b>45-0513</b>	Petri, 7 mm, Tissue Chamber Kit	1 Ea
<b>45-0505</b>	Petri Dish Tissue Chamber, 5 x 5 mm Kit	1 Ea
<b>45-0504</b>	Petri Dish Tissue Chamber, 5 x 5 mm (needs 45-0216)	1 Ea
<b>45-0506</b>	Petri Dish Tissue Chamber, 15 x 15 mm (needs 45-0216)	1 Ea
<b>45-0507</b>	Petri Dish Tissue Chamber, 15 x 15 mm Kit	1 Ea
<b>45-0100</b>	Petri Dish Electrode, 2 mm gap, 90 mm Well Dish Kit	1 Ea
<b>45-0130</b>	Petri Pulser for 6 Well Plates, 35 mm Well Kit	1 Ea
<b>45-0490</b>	Tissue Slice Chamber, 7 x 7 mm Kit	1 Ea

Order No.	Description	UOM
<b>45-0491</b>	Tissue Slice Chamber, 7 x 7 mm Dish (needs 45-0492, 45-0503, 45-0204)	1 Ea
<b>45-0492</b>	Tissue Slice Wand, (+) 7 mm (needs 45-0491, 45-0503, 45-0204)	1 Ea
<b>45-0500</b>	Tissue Slice Chamber, 10 x 10 mm Kit	1 Ea
<b>45-0501</b>	Tissue Slice Chamber, 10 x 10 mm Dish (needs 45-0502, 45-0503, 45-0204)	1 Ea
<b>45-0502</b>	Tissue Slice Wand, (+) 10 mm (needs 45-0501, 45-0503, 45-0204)	1 Ea
<b>45-0503</b>	Mini Micro Grabber Adapter Cables for Tissue Slice Chamber / L Shaped Needle Electrodes	1 Ea
<b>45-0530</b>	Adherent Cell Electrode, 3 mm gap (needs 45-0204)	1 Ea
<b>45-0531</b>	Adherent Cell Electrode, 3 mm Kit	1 Ea
<b>45-0486</b>	PT Tweezertrodes, 1 mm Diameter Kit	1 Ea
<b>45-0487</b>	PT Tweezertrodes, 3 mm Diameter Kit	1 Ea
<b>45-0489</b>	PT Tweezertrodes, 5 mm Diameter Kit	1 Ea
<b>45-0488</b>	PT Tweezertrodes, 7 mm Diameter Kit	1 Ea
<b>45-0165</b>	SS Tweezertrode, 7 mm Diameter Kit	1 Ea
<b>45-0118</b>	SS Tweezertrode, 7 mm Diameter (needs 45-0204)	1 Ea
<b>45-0166</b>	SS Tweezertrode, 10 mm Diameter Kit	1 Ea
<b>45-0119</b>	SS Tweezertrode, 10 mm Diameter (needs 45-0204)	1 Ea
<b>45-0524</b>	PT Tweezertrode, 1 mm Flat (needs 45-0204)	1 Ea
<b>45-0525</b>	PT Tweezertrode, 1 mm Flat Kit	1 Ea
<b>45-0204</b>	Tweezertrode Cable / Single Adaptor Cable for Tissue Slice Electrode Positive	1 Ea
<b>Specialty Electrodes (AgilePulse Systems Only)</b>		
<b>47-0090</b>	Electrode Adapter Box for Agile Pulse <i>In Vivo</i>	1 Ea
<b>47-0000</b>	Parallel-Needle Array Handle for AP <i>In Vivo</i>	1 Ea
<b>47-0040</b>	4-Needle Array, 4 mm gap, 2 mm Length, AP <i>In Vivo</i> (ID*)	1 Ea
<b>47-0043</b>	3-Needle Array, 4 mm gap, 3 mm Length, AP <i>In Vivo</i> (IM)	1 Ea
<b>47-0045</b>	4-Needle Array, 4 mm gap, 5 mm Length, AP <i>In Vivo</i> (IM*)	1 Ea
<b>47-0050</b>	6-Needle Array, 4 mm gap, 2 mm Length, AP <i>In Vivo</i> (ID*)	1 Ea
<b>47-0060</b>	6-Needle Array, 6 mm gap, 2 mm Length, AP <i>In Vivo</i> (ID)	1 Ea
<b>47-0070</b>	6-Needle Array, 6 mm gap, 10 mm Length, AP <i>In Vivo</i> (IM*)	1 Ea
<b>47-0080</b>	6-Needle Array, 6 mm gap, 12 mm Length, AP <i>In Vivo</i> (IM)	1 Ea
<b>47-0086</b>	6-Needle Array, 6 mm gap, 16 mm Length, AP <i>In Vivo</i> (IM)	1 Ea
<b>47-0204N</b>	5 ml Chamber for Agile Pulse Max	1 Ea
<b>47-0090</b>	Electrode Adapter Box for Agile Pulse <i>In Vivo</i>	1 Ea
<b>47-0206</b>	10 ml Chamber for Agile Pulse Max	1 Ea

# Declaration of Conformity

Manufacturer: *Harvard Apparatus, Inc.  
84 October Hill Road  
Holliston, Massachusetts 01746-1388, U.S.A.  
Phone: (508) 893-8999*

We herewith declare that the following product:

Product Name: *Gemini X<sup>2</sup> Gemini SC<sup>2</sup>*

To which this declaration relates, is in conformity with the applicable EC Directives, harmonized standards, and other normative requirements:

Application of  
Council Directive(s): *2006/95/EC Low Voltage Directive  
2004/108/EC Electromagnetic Compatibility Directive  
RoHS Compliant*

Standard(s) to which conformity is declared:

Safety: *EN 61010-1:2012 (3rd Edition)*  
Emissions/Immunity: *EN 61326:2006/05/01  
FR47 FCC Part 15 Sub Part B (2013)  
EN 61000-4-2:2001  
EN 61000-4-3:2002  
EN 61000-4-4:2004  
EN 61000-4-5:2001  
EN 61000-4-6:2003  
EN 61000-4-8 1993+A1:2000  
EN 61000-4-11:2004  
EN 61000-3-2:2000  
EN 61000-3-3:2002  
CISPR11:2003+A1:2013, Class A*

EMC and Safety compliance were evaluated by Intertek/ETL Semko

Reference test report  
file numbers: *101111687 BOX-001, -002, -003, -004, -005*

I, the undersigned, hereby declare that the equipment specified above conforms to the above Directive(s) and Standard(s).

Place: *United States of America*  
Date: *April 23, 2013*



**HARVARD**  
APPARATUS

*Beth Bauman*  
(Signature)

Beth Bauman  
(Full Name)

VP Engineering / Operations  
(Position)