

ECM 830

Square Wave Electroporation System

USER'S MANUAL



BTX[®]

a division of **Harvard Bioscience, Inc.**

Publication 5423-025-REV 1.1

Warranty Information

Research Only

BTX

84 October Hill Rd
Holliston, MA 01746, USA

Phone: 1-508-893-8999

Fax: 1-800-429-5732

Web: www.btxonline.com

Warranty

BTX warrants the ECM 830 Electroporation Systems for a period of two years from the date of purchase. At its option, 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 BTX without BTX express and prior approval, (c) used in violation of instructions furnished by BTX. This warranty extends only to the original customer purchaser. **IN NO EVENT SHALL 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, 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 ECM 830 instrument is changed in any way from its original factory design or if repairs are attempted without written authorization by BTX. Warranty is void if parts, connections or electrodes not manufactured by BTX are used with the ECM 830 instrument. If a defect arises within the warranty period, promptly contact 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: support@hbiosci.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.

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 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 BTX technicians.

Repair Facilities and Parts

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 ECM 830 system is 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) and safety intended for laboratory equipment applications.

This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.

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 ECM 830 system 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 three 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.

Observe All Warning Labels on Product

Read all labels on product to ensure proper usage.

High Voltage Risk

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 BTX Customer service at 1-800-272-2775, or 1-508-893-8999.

Caution Notice

The ECM 830 Square Wave Electroporation System is 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 as well as the applicable safety requirements for electrical equipment for measurement, control, and laboratory use. The unit itself does not generate waste, but may be used to treat samples that are hazardous. Please use appropriate PPE and ensure disposal in accordance with local regulations and practices.

This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.



Caution



Protective Ground Terminal



Caution Risk of Electric Shock

A Message from BTX

Thank you for investing in a ECM 830 System.

Since its founding in 1983, the main focus of BTX 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 waveforms, electrodes and chamber options to provide you with the best tools to achieve your goals.

We are vested in your success. To that end, the 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:

BTX

84 October Hill Road
Holliston, MA 01746 USA

Toll Free: 1-800-272-2775

International Callers: 508-893-8999

Fax: 508-429-5732

Web: www.btxonline.com

Email: support@hbiosci.com

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



a division of **Harvard Bioscience, Inc.**

Table of Contents

SUBJECT	PAGE #
Warranty Information	2
Safety Information	3
Introduction	4
Table of Contents	5 – 6
Product Overview	7
Unpacking the System	8
Touchscreen Button Reference	9
Software Setup	10 – 12
Setting Time/Date	10
Setting Audible Alarm Preferences	10
Setting Backlight Preferences	11
Displaying Device Information	11
Toggling Auto-PrePulse	12
Preset Protocols	13 – 15
Using Preset Protocols	13
Customizing a Preset Protocol	14 – 15
Performing Experiments	16 – 18
Creating New Protocols	16 – 18
Using Specialty Protocols	19 – 21
Using an HT Plate Handler	19 – 20
Using Specialty Electrodes	21
Managing Protocols	22 – 24
Saving a Copy of a Protocol	22
Renaming a Protocol	22
Deleting a Protocol	23
Password Protecting a Protocol	24
Protocol Manager Software	25 – 27
Installation	25
Overview	25
Upload from Generator to PC	25
Download from PC to Generator	26
Upload Log Files	27
Remote Control Software	28
Generator Specifications	29
Maintenance	30
Upgrading ECM 830 Series Software	31 – 33
USB Virtual CommPort Driver Installation	31 – 32
Firmware Updater Program	33
Error Messages & Troubleshooting	34 – 36

Table of Contents

SUBJECT	PAGE #
General Optimization Guide for Electroporation	37 – 39
DNA Delivery into Cells Using Electroporation	37
Waveforms	38
Field Strength	38
Pulse Length	39
Number of Pulses	39
Electroporation Buffer	39
DNA/RNA Concentrations	39
Applications	40
Mammalian Cell Transfection	40
In Vivo, In Utero, In Ovo	40
Electroporation Buffers	41
Ordering Information	42 – 44

Product Overview

The ECM 830 System is a versatile electroporation system suitable for applications for gene, drug and protein delivery including: CRISPR, mammalian cells, in vivo, ex vivo, in ovo tissue, ex plant and nuclear transfer.

The ECM 830 is a square wave pulse generator designed for in vitro and in vivo applications. The versatility of the ECM 830 System applications for gene, drug and protein delivery include; mammalian cells, in vivo and ex vivo tissue, zebrafish and embryos, nuclear transfer, embryo manipulation, plant protoplast and basic bacteria and yeast transformations. All accessories plug directly into the unit.

The ECM 830 can be used in combination with a wide array of BTX specialty electrodes and accessories to enhance your molecular and drug delivery experiments.

Features

- A wide range of voltages from 5 to 3,000 V
- Finer voltage discrimination
- Pulse durations from 10 μ s to 1 s
- Arc detection
- Digital display of actual voltage and pulse length delivered

Applications

- Mammalian cell transfection
- In vitro, in vivo, ex vivo and in ovo tissue transfection
- Nuclear transfer
- Ex plant tissue transfection
- Plant protoplast transfection
- siRNA libraries
- High throughput 25- and 96-well electroporation

High Throughput (HT) Electroporation

The High Throughput (HT) 96- and 25-well systems offer the advantage of multi-well electroporation technology for processing multiple samples in seconds. Using the HT Multi-Well plates instead of traditional cuvettes, the researcher can transition from a single cuvette to either a 96-well or 25-well electroporation plate by using a plate handler. This increases yields and the number of experiment runs in a single day. Experiments take seconds to run, allowing for quick and efficient optimization of the electrical and biological parameters. Once optimized, samples are rapidly processed increasing yields and saving valuable time and money.

The key to the HT System is the combination of the ECM 830 Generator with the Plate Handler and multi-well plates. The Plate Handler holds the plates and delivers the pulse(s) to the wells using parameters set in the ECM 830 generator.

Monitoring Option

The Enhancer 3000 allows the researcher to monitor and track key electrical parameters used in electroporation applications. The electrical pulse data is captured as both a graphic display of the waveform and electrical output values following each electroporation experiment.



CAUTION: FOR RESEARCH USE ONLY. NOT FOR CLINICAL OR DIAGNOSTIC USE ON PATIENTS.

Unpacking the System

The shipping carton in which your ECM 830 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 ECM 830 system 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 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.

Remove the insulating tab from the battery at the base of the unit.

Place the generator in a location that is a dry, level, sturdy surface free from extremes in ambient temperature, dust or chemical exposures. Allow the device to equilibrate to room temperature.

Unpack the safety dome, cuvette rack and disposable cuvette chambers. Connect the safety dome, specialty electrode, or the HT plate handler into the connectors at the bottom right-hand side of the 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 Main Menu screen will appear.

You are now ready to begin your work.



Remove Battery Insulating Tab.



Touchscreen Icons 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.



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.



Scroll Down

Used to scroll down in a display list.



Scroll Up

Used to scroll up in a display list.



Pre-Pulse Resistance Measurement

Also referred to as Omega Icon. 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.



Settings

Also referred to as Gear icon. 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.



Protocol Locked

Indicates that changes to the protocol are currently locked. Press the icon and enter password to unlock the protocol.



Protocol Unlocked

Indicates that changes to the protocol are currently unlocked. Pressing the icon twice will allow the user to password protect the protocol.

Notification Box Quick Reference



Information / Status Message

Provides information or guidance for next steps, including acceptable ranges for protocol programming.



Warning Message

Requires Notification Box tap for acknowledgement of information, but does not prevent operation of the system.



Failure Message

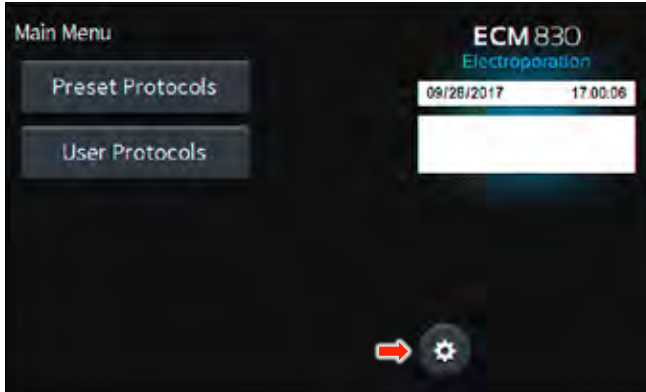
Caused by user input error, failure of a protocol, or a system error. Requires user to correct this issue in order to complete the operation.

Software Setup

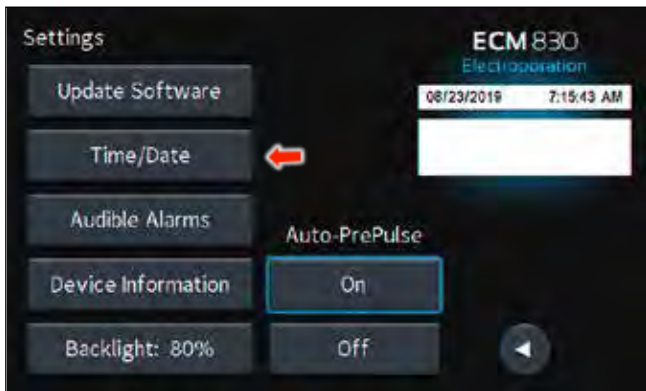
Setting Time/Date

After initializing, the home screen will appear.

Tap the **Gear** 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.

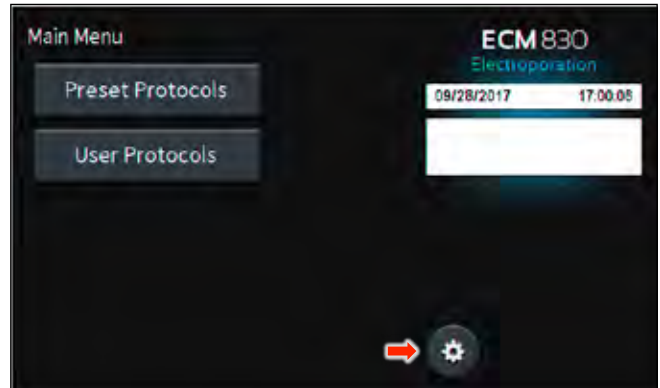
Tap the **Green Check** icon to save and return to the settings screen.

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

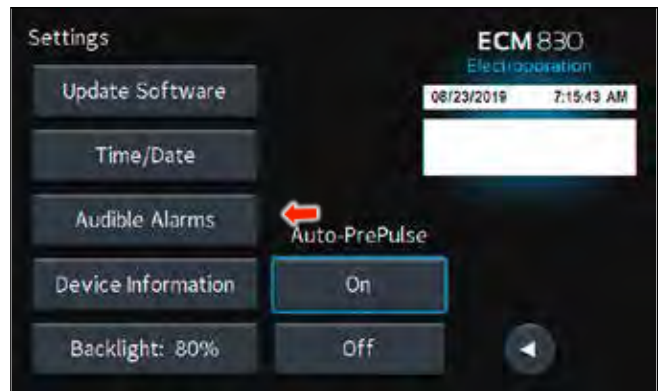


Setting Audible Alarm Preferences

On the home screen, tap the **Gear** icon.

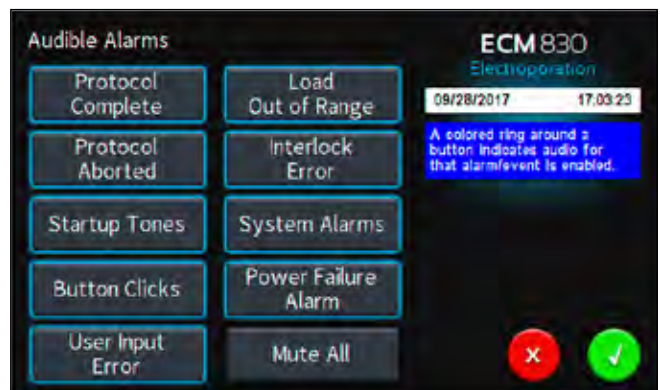


Tap **Audible Alarms** icon.



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

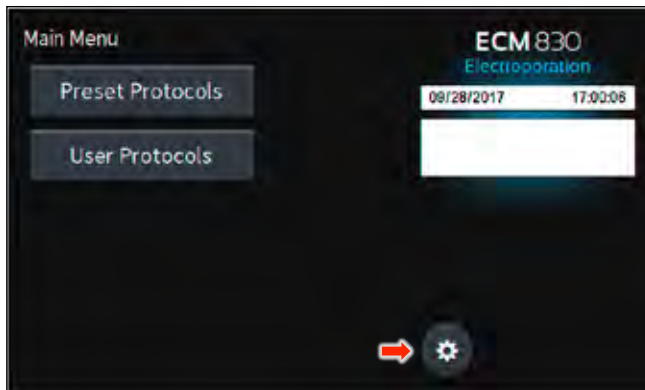
Tap the **Green Check** icon to save and return to the settings screen.



Software Setup

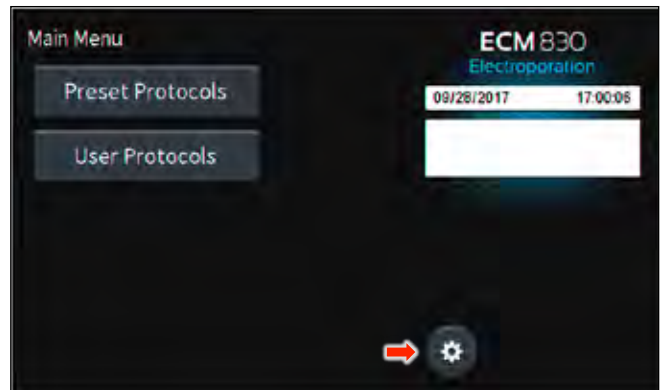
Setting Backlight Preferences

On the home screen, tap the **Gear** icon.

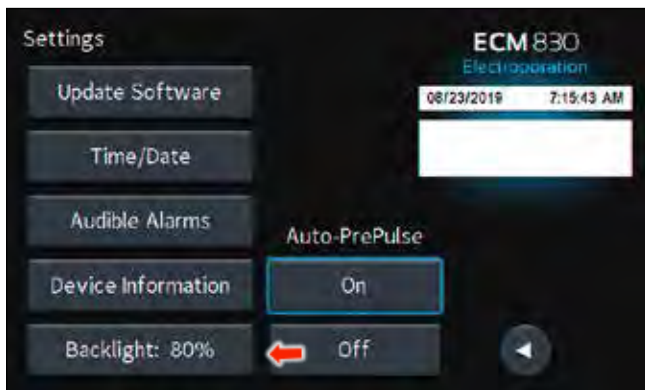


Displaying Device Information

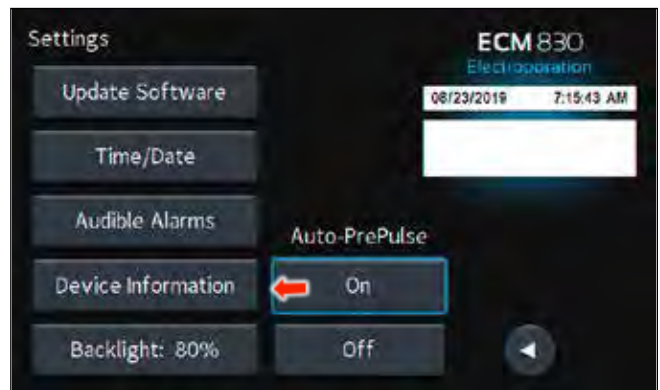
On the home screen, tap the **Gear** icon.



Tap **Backlight** icon.



Tap **Device Information** icon.



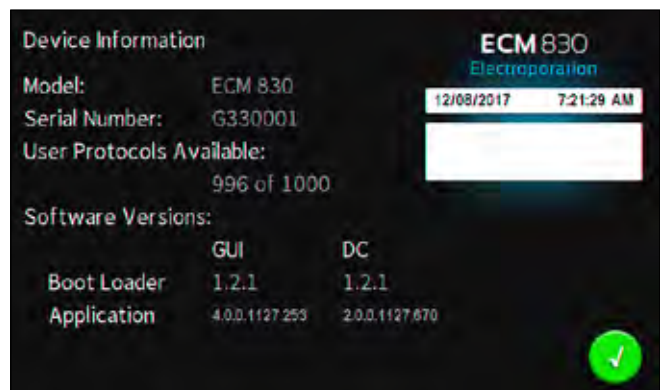
Select preferred brightness by tapping the icon indicating % brightness.

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



View device information.

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

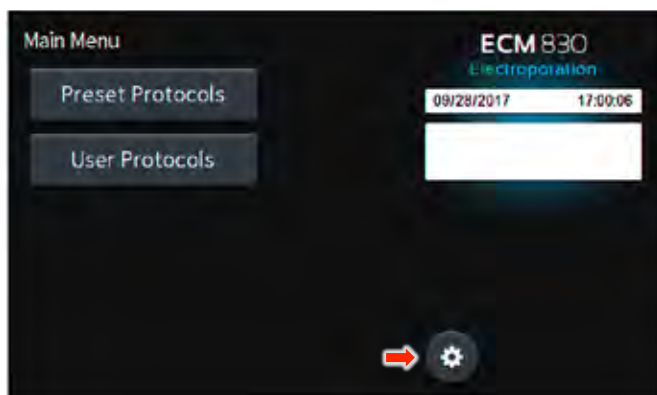


Software Setup

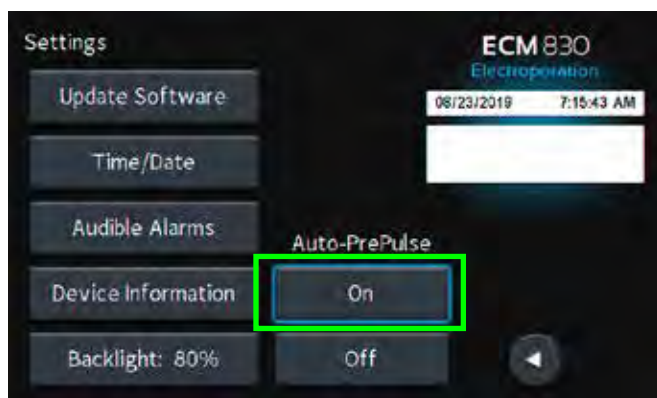
Toggleing Auto-PrePulse Off/On

NOTE: Default instrument settings include an automatic square wave PrePulse of approximately 17 volts, 30 ms duration executed prior to protocol programmed pulse(s) to measure sample resistance and configure the equipment prior to running. When a PrePulse runs, a PrePulse instrument log is recorded which includes Date, Time, Model, Mode, Protocol Name, and Sample Resistance ("Load," in Ohms). The PrePulse log file name is the same as an experimental pulse log file name, except with a PP appended to the end, for example "222412PP.txt."

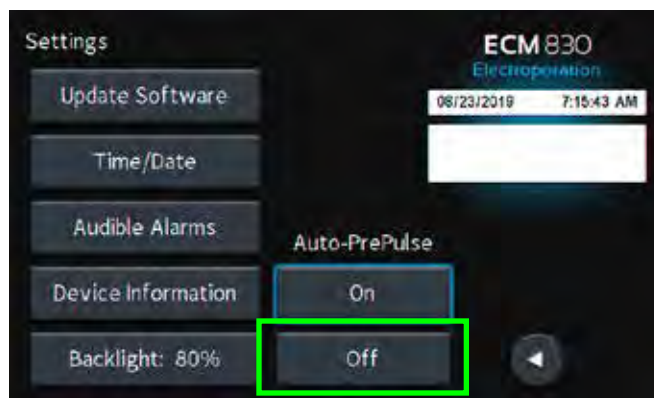
From the main menu or the protocol run screen, tap the Gear icon to go to Settings.



Auto-PrePulse default setting is **On**.



Auto-PrePulse may be deactivated by tapping Off icon or activated by tapping On icon. Selected Auto-PrePulse settings are maintained if instrument is power cycled.

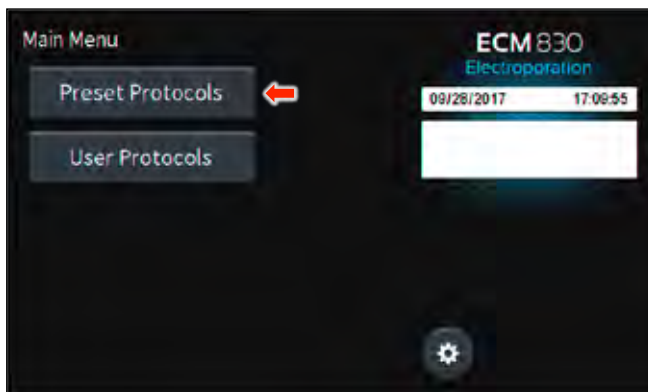


NOTE: When Auto-PrePulse is disabled, sample resistance is no longer recorded in the experimental log. In both situations whether Auto-PrePulse is enabled or disabled, the user has the option to manually run a Pre-Pulse sample resistance check and log PrePulse data. To run a manual PrePulse, tap the Ohm icon on the protocol run screen. This allows the user the ability to perform a manual sample resistance PrePulse check and log PrePulse data on a duplicate sample separate from an experimental sample protocol run with the Auto-PrePulse feature disabled.

Preset Protocols

Using Preset Protocols

On the home screen, tap the **Preset Protocols** icon.



Double tap desired cell type.



Scroll through available cells.

Tap desired cells.



Using Preset Protocols (continued)

Review parameters.

Tap **Omega** icon to measure pre-pulse load resistance.



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



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

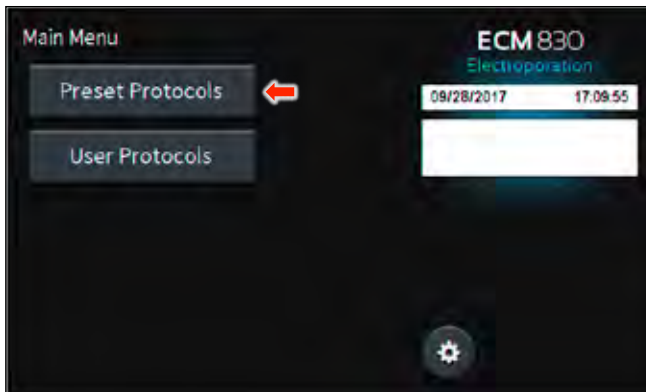
You may continue pulsing, go back to the protocol select screen, or to the home screen.



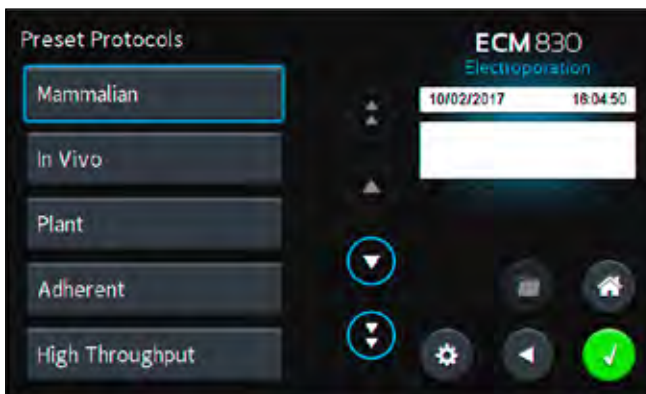
Preset Protocols

Customizing a Preset Protocol

On the home screen, tap the **Preset Protocols** icon.



Double tap desired cell type.



Scroll through available cells.

Tap desired cells.

Tap **Folder** icon.



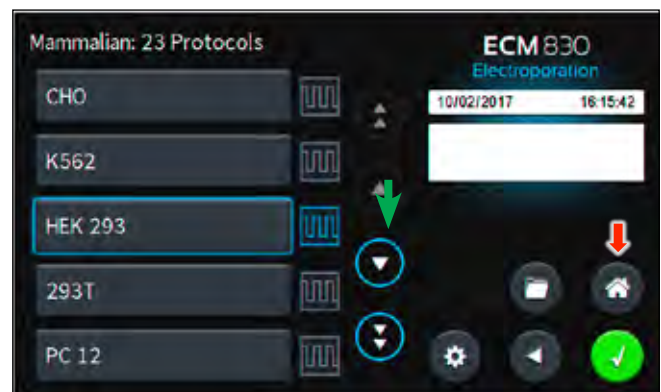
Customizing a Preset Protocol (continued)

Use keyboard to name copy of protocol.

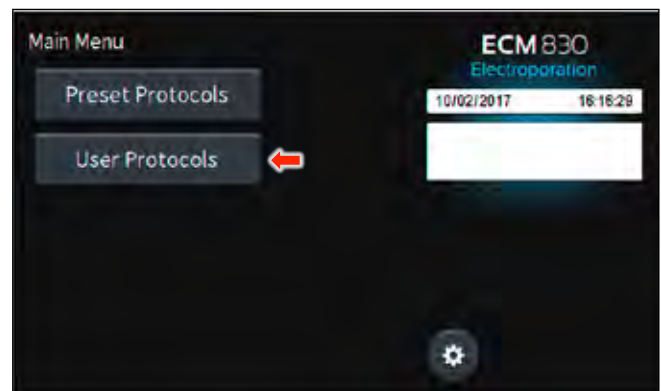
Tap the **Green Check** icon to save.



Tap the **Home** icon.



On the home screen, tap **User Protocols** icon.



Preset Protocols

Customizing a Preset Protocol (continued)

Scroll through user protocols to locate the newly saved method.

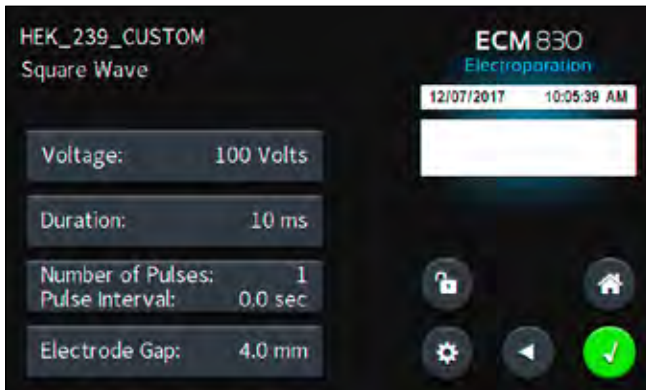
Double tap the newly saved method.



Tap the parameter(s) requiring customization.

Proceed to modify selected parameters.

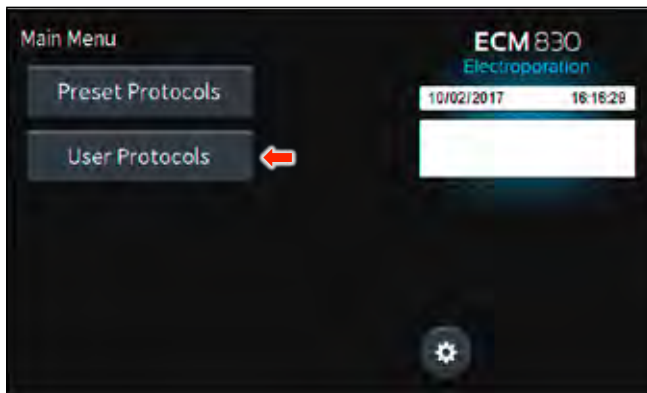
Tap the **Green Check** icon to proceed to the run screen.



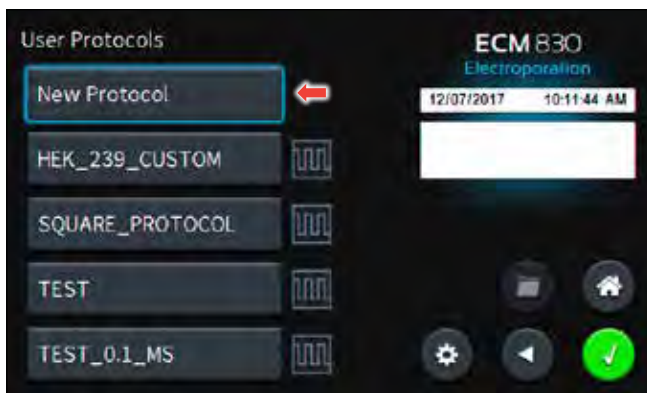
Performing Experiments

Creating New Protocols

On the home screen, tap **User Protocols** icon.



Double tap **New Protocol**.



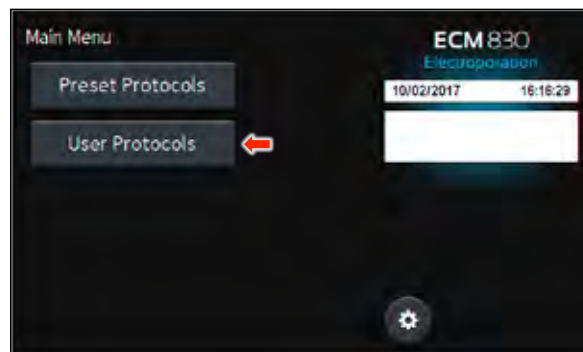
Use keyboard to name protocol.

Tap the **Green Check** icon to save.



Creating New Protocols

On the home screen, tap **User Protocols** icon.



Tap your newly saved protocol.

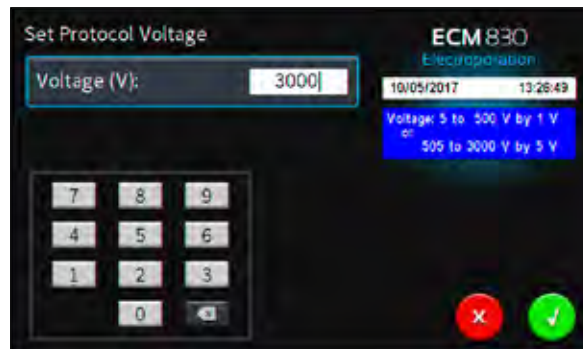


Tap the **Voltage** icon.



Use the numeric keypad to set voltage.

Tap the **Green Check** icon to save.



Performing Experiments

Creating New Protocols (continued)

Tap the **Duration** icon.



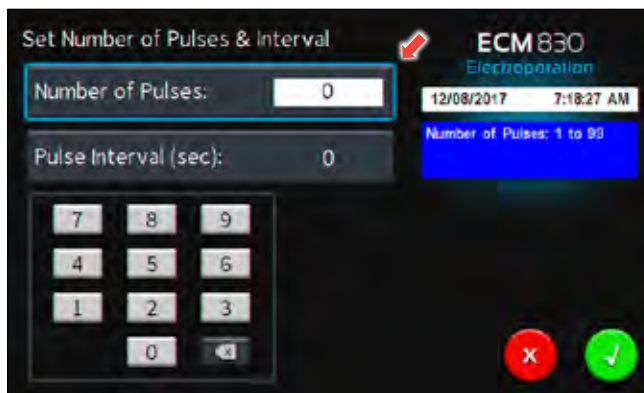
Use the numeric keypad to set duration of pulse.



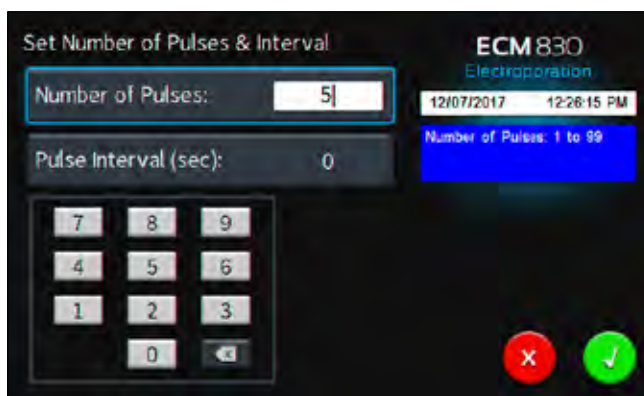
Tap **Number of Pulses / Pulse Interval** icon.



Tap the **Number of Pulses**.



Use the numeric keypad to set the # of pulses.



Tap the **Pulse 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

Creating New Protocols (continued)

Tap the **Electrode 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.



View your protocol.

To run, tap the **Omega** icon.



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



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 set-up screen, or to the home screen.

NOTE: See the Protocol Manager Software section for instructions on viewing and downloading log data.



Using Specialty Protocols

Using an HT Plate Handler

Plug the HT Plate Handler into the front ports of the ECM 830 unit.

Select desired protocol (preset protocol or user protocol).

Double tap desired cells.



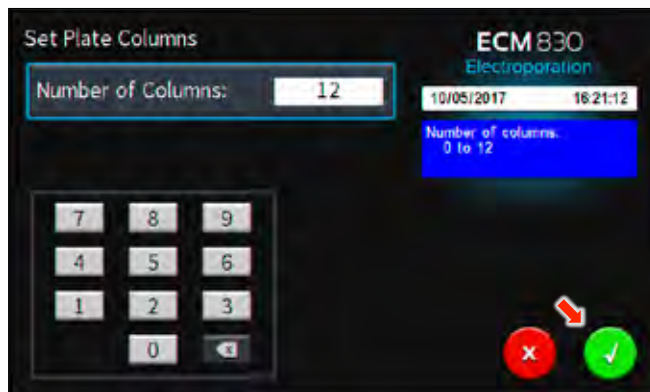
Review parameters.

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 on 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 green **Information Screen** to clear message.

Tap the **Green 'Go'** icon to run protocol.



The **Red 'x'** 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.



Using Specialty Protocols

Using an HT Plate Handler (continued)

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.



Review parameters.

Position electrodes on area being electroporated.

To run tap the **Omega** icon.



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

Using Specialty Electrodes

Plug the specialty electrodes into the front ports of the BTX ECM 830.

Scroll through available protocols.

Double tap desired selection.



Scroll through available tissue/cell types.

Double tap desired tissue/cell.



While the pulse is being delivered, the **Red 'x'** icon can be used to stop the protocol at any time.

NOTE: Generator will display readings after first and last pulse.



Using Specialty Protocols

Using Specialty Electrodes

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

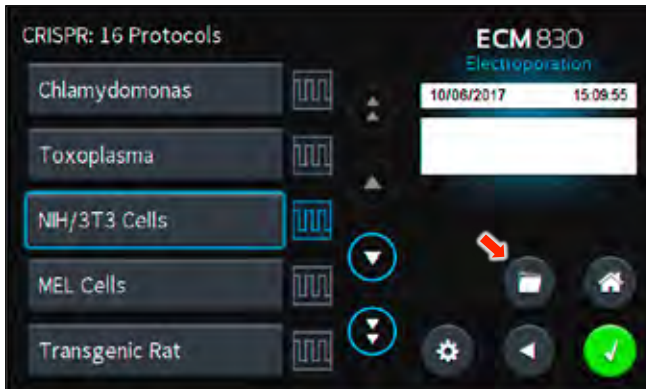
You may continue pulsing, go back to the settings screen or the protocol select screen, or to the home screen.



Managing Protocols

All preset and user 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 ecm 830 allows you to export protocol information to an externally connected generator or to a computer that will be used to control operation. This section describes the file management facilities available on the ECM 830.

Preset protocols cannot be edited or deleted but may be saved as a new user protocol where they can be edited, deleted or renamed.



Saving a Copy of a Protocol

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

1. From the list of available Protocols, select (press once) the Protocol you want to save a copy of, then choose the **File Options** icon to enter the File Options screen.

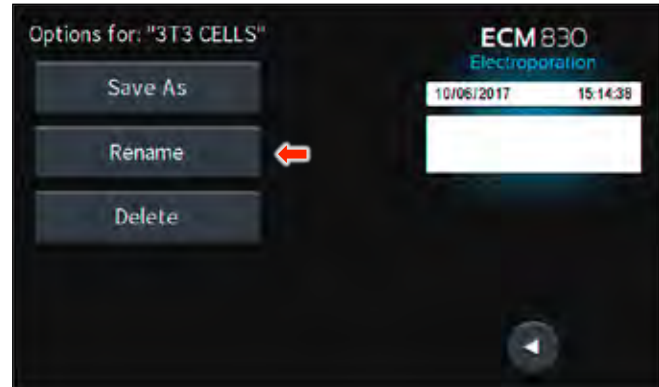
NOTE: If saving a **Preset Protocol** as a new **User Protocol**, the **File Options** icon will directly open up to the **Protocol Name** screen.

2. Choose **Save As/Copy** to access the **Protocol Name Entry** screen. The current protocol name will be displayed in the text entry box.
3. On the **Protocol Name** screen, type the new name for your protocol using the onscreen keyboard. Choose the **Symbols / Numbers** icon (.@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** icon (ABC). [15 characters maximum]
4. When finished entering the new Protocol Name, choose the **Green Check** icon to save your changes.

Renaming a Protocol

Follow these instructions to rename a previously saved user protocol:

1. From the list of available Protocols, select (press once) the Protocol you want to rename, then choose the **File Options** icon to enter the **File Options** screen.



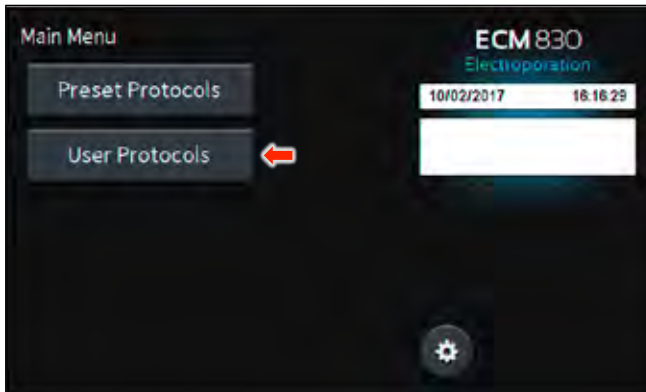
2. Choose **Rename Protocol** to access the **Protocol Name Entry** screen.
3. On the **Protocol Name** screen, type the new name for your protocol using the onscreen keyboard. Choose the **Symbols / Numbers** button (.@123) to display the list of available numbers and symbols that can be included in the protocol name. To switch the keyboard back to letters mode, choose the **Letters** icon (ABC). [15 characters maximum]
4. When finished entering the new protocol name, choose the **Green Check** icon to save your changes.

Managing Protocols

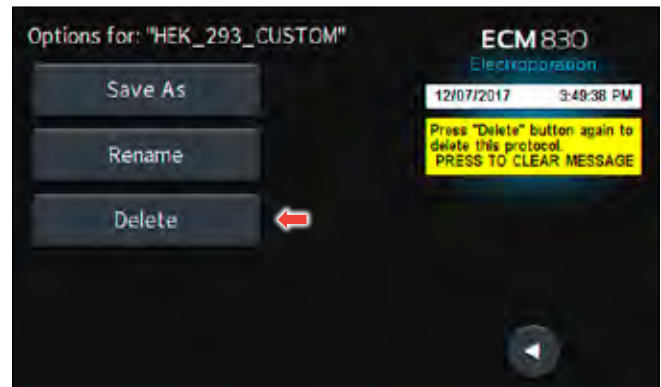
Deleting a Protocol

NOTE: Preset protocols cannot be deleted.

On the home screen, tap the **User Protocols** icon.



Confirm delete by tapping the **Delete** icon again.



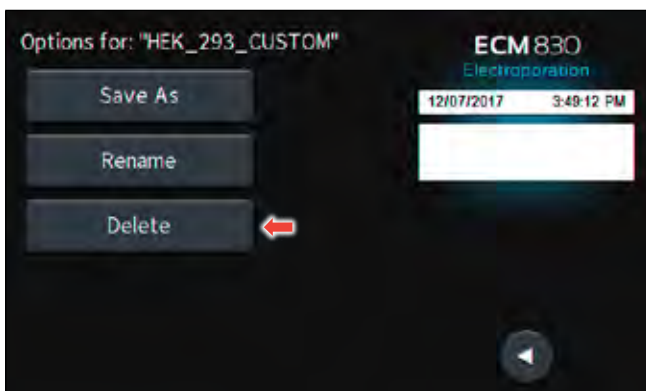
Protocol has been removed from the menu.

Tap the protocol you wish to delete.

Tap **Folder** icon.



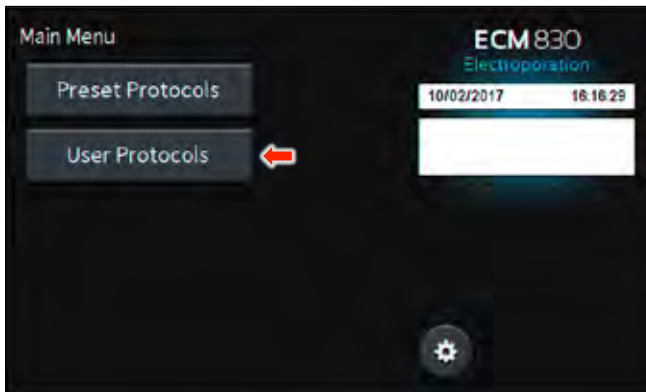
Tap **Delete** icon.



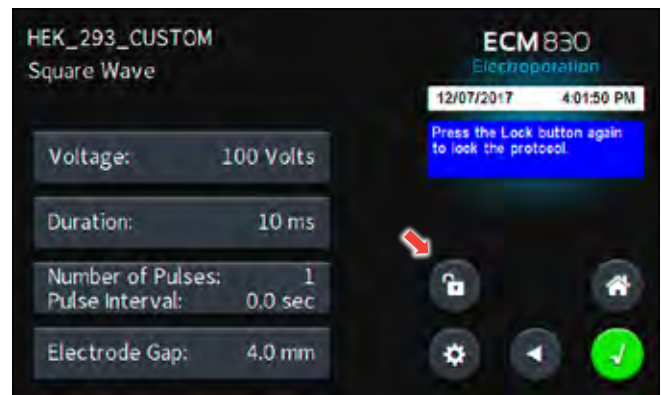
Managing Protocols

Password Protecting a User Protocol

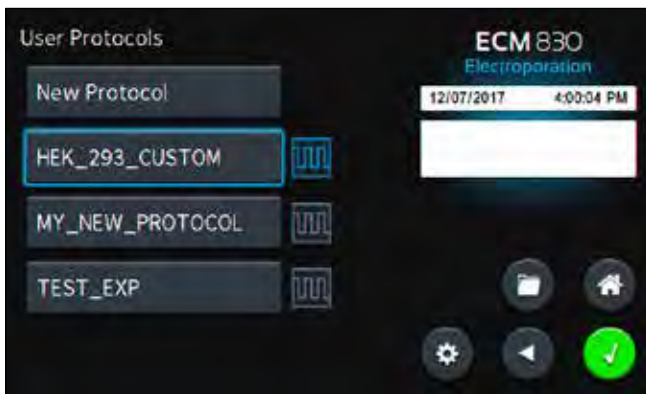
On the home screen, tap the **User Protocols** icon.



Confirm password protection by tapping the **Open Lock** icon again.

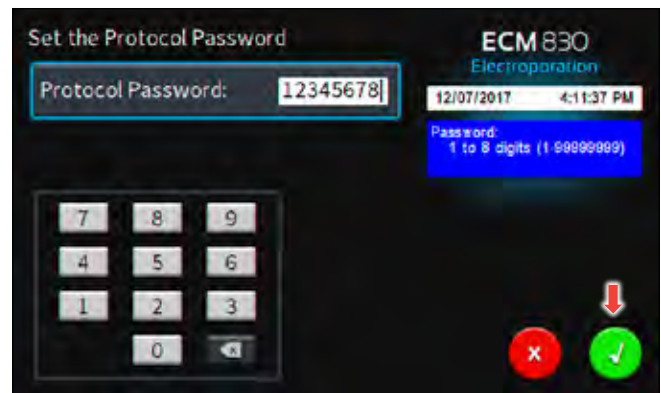


Double tap protocol you wish to password protect.



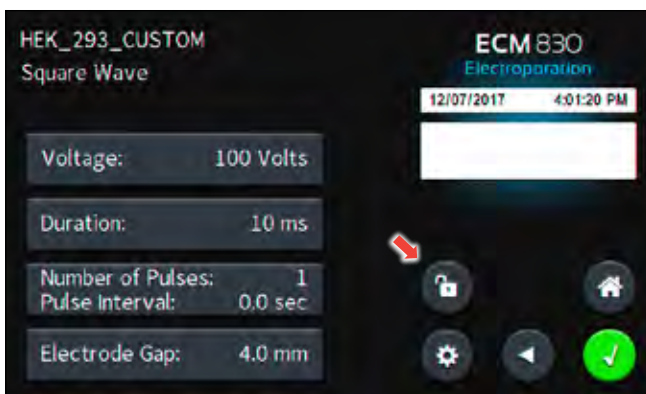
Use the numeric keypad to set the password.

Tap the **Green Check** icon to save.



View the protocol.

Tap the **Open Lock** icon.



The protocol is now locked against modifications.

To unlock press the **Lock** icon. Type in the password then tap the **Green Check** icon.



Protocol Manager Software

Installation

Download the software from BTX website www.btxonline.com. Click on **Technical Resources** drop-down menu and select **Downloads**. Download and install file named **Protocol Manager**.

NOTE: Compatible with Windows® XP or later.

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

Overview

Protocol Manager allows the user to:

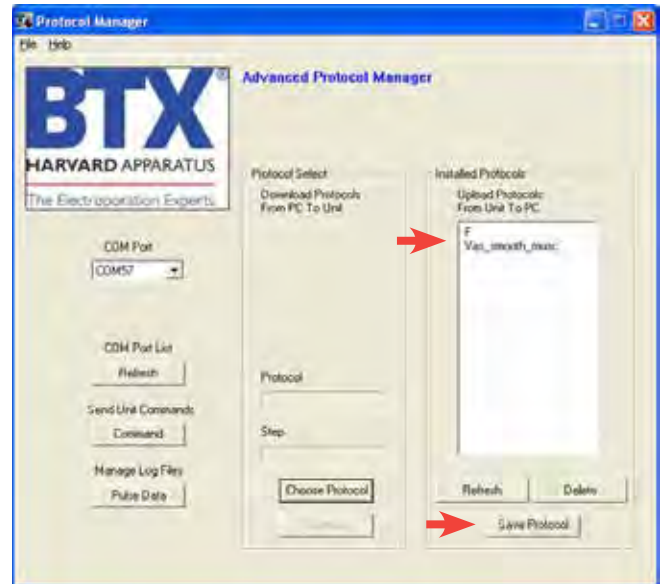
- Upload protocols from the ECM 830 generator to a PC.
- Download protocols from a PC to the ECM 830 generator.
- Upload log files from the ECM 830 generator to a PC.



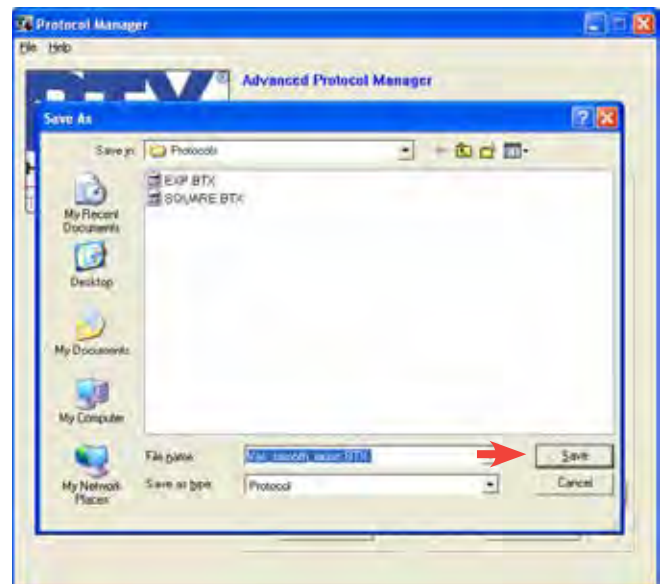
Upload from Generator to PC

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

NOTE: Hit 'Refresh' to update the list after creating or editing protocols on the ECM 830 generator.



3. Select the drive and folder location.
4. Click 'Save'.

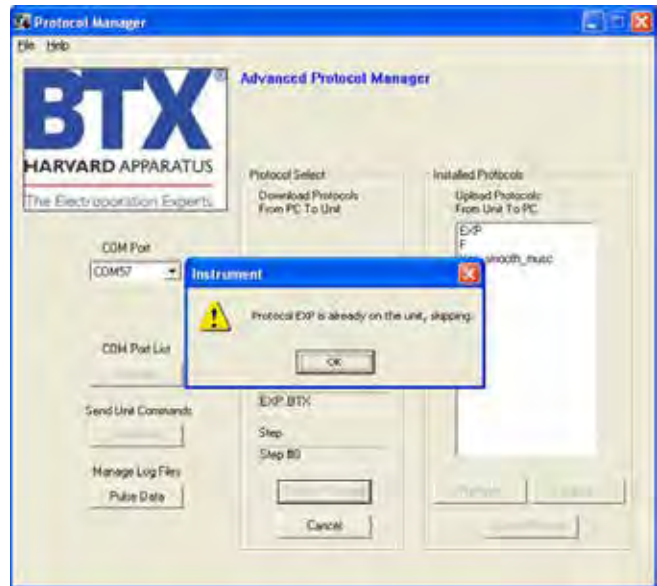
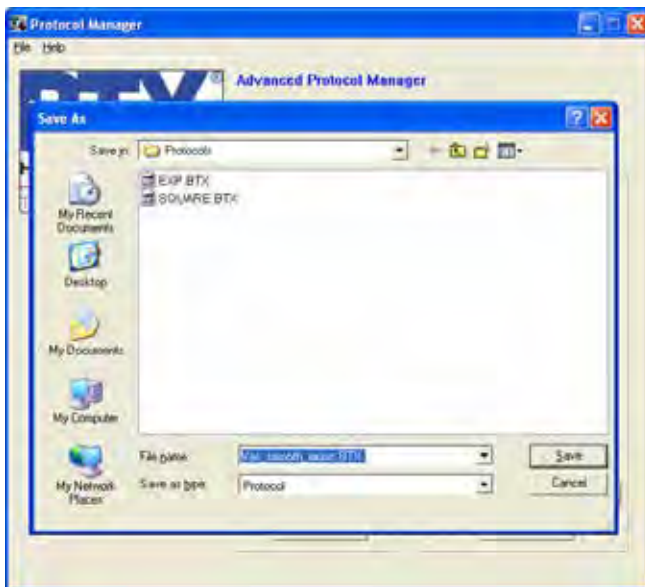


Protocol Manager Software

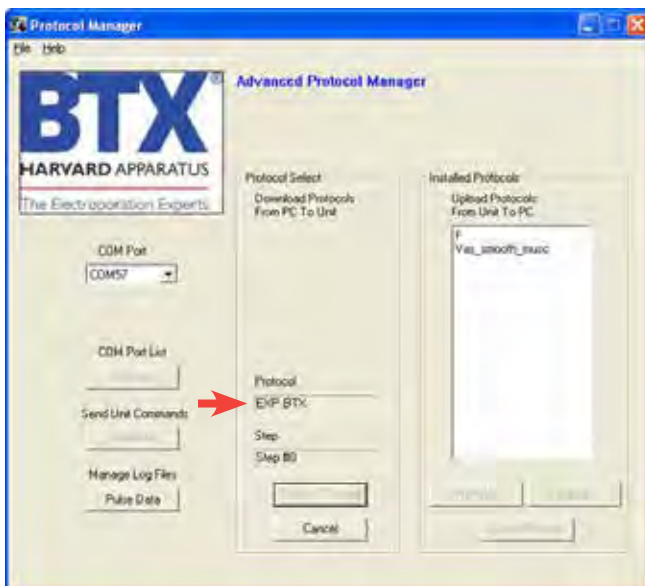
Download from PC to Generator

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

NOTE: If a protocol already exists on the ECM 830 generator, Protocol Manager will not overwrite the file if you attempt to re-download it.



4. Protocol name being downloaded is shown.

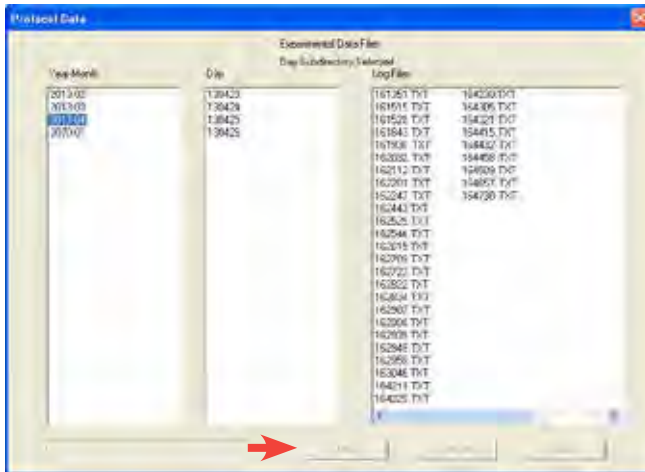


Protocol Manager Software

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.

NOTE: Log Files are saved as a tab delimited text file. They can be opened with any common text editor like Notepad or by using spreadsheet software such as Excel.



Remote Control Software

Installation

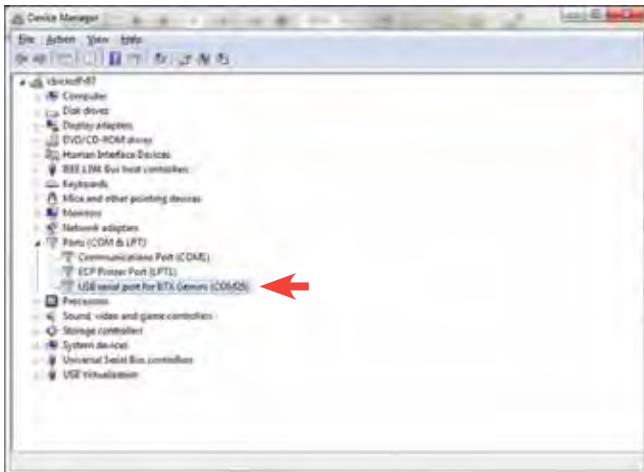
Download the software from the BTX website www.btxonline.com. Click on **Technical Resources** drop-down menu and select **Downloads**. Download and install file named **Remote Screen Interface**.

NOTE: During the installation, it will ask you to provide a location to install your application files. There is a known issue with this installer where it will not actually create this directory.

Overview

Remote Screen Interface allows you to control the ECM 830 remotely. When you click on the computer screen, you control the generator.

While the ECM 830 generator is connected 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. Under **Ports**, search for the 'USB serial port for ECM 830.' This will show the COM Port to which the generator is connected, as shown below.



Once the COM port is known, open the Remote Screen Interface application. Choose the correct COM port and click 'Connect'.



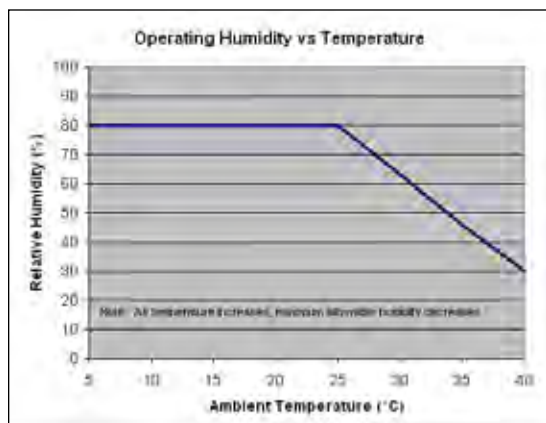
You should now be able to control your ECM 830 generator remotely.



Generator Specifications

ECM 830	
Square Wave Pulse Ranges	
Voltage Range	
LV Mode	5 to 500 in 1 V steps
HV Mode	505 to 3000 in 5 V steps
Voltage Accuracy	
LV Mode	5%
HV Mode	5%
Pulse Length Range	
LV Mode	10 to 999 μ s in 1 μ s step 1 to 999 ms in 1 ms step
HV Mode	10 to 600 μ s in 1 μ s step
Multiple Pulsing	
LV Mode	1 to 99 pulses per sample
HV Mode	1 to 99 pulses per sample
Pulse Interval	0.1 s to 10 s
Capacitance in μF	
LV Mode	3775 μ F
HV Mode	85 μ F
Voltage Droop	20% Load <1 K Ω , 10% Load >1 K Ω
Sample Resistance (Load)	
LV Mode	PL \leq 100 ms, load \geq 8 to 9 Ω PL >100 ms, load \geq 100 Ω
HV Mode	Load \geq 40 Ω
Other Specifications	
Charging Time	LV <7 s, HV <4 s
Monitoring	Pulse Voltage, Width, Droop % and Sample Resistance
Display	7.0-in color display
Controls	Touch Screen
Programmability	Storage over 1000 Protocols
Arc Control	Yes

Other Specifications	
Interlock Switches	Yes
Pre-Pulse Sample resistance check	Yes
Pulse Over Current Protection	Yes
PC control	Yes
PC communications	Yes
Log Report	Yes
Remote Operation	Yes
Foot Switch Control	Yes
Power Ratings	35 W idle and 350 W pulsing
Input Voltage ratings	100 to 240 VAC
Dimensions (H x W x D) inches	12.75 x 11.25 x 8.5
Weight	~16 lb
Warranty	2 years
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	20% to 80% RH, non-condensing
Storage Humidity	20% to 80% RH, non-condensing
Mode of Operation	Continuous
Classification	Class I
Pollution	IP2X
Installation	Category II
Supplier Name	BTX
Supplier Address	84 October Hill Rd. Holliston, MA 01746
Supplier Phone Number	508-893-8999
Regulatory Certifications	CE, ETL (UL, CSA), FCC, WEEE, EU RoHS & CB Scheme



Maintenance

The ECM 830 generator requires no special maintenance other than keeping it clean.

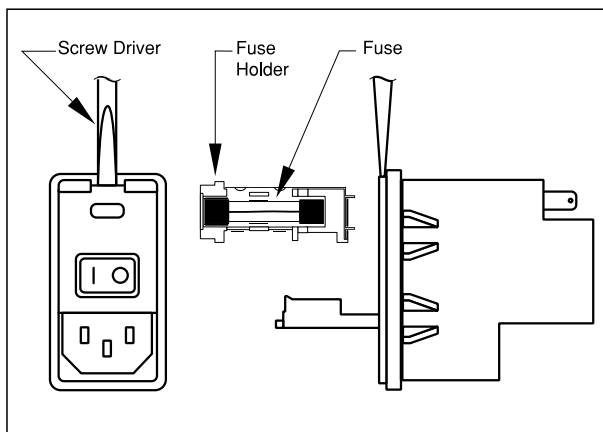
To clean the exterior surfaces, use a lint-free cloth to remove loose dust. Use care to avoid scratching the clear display window. For more efficient cleaning, use a soft cloth dampened (not soaked) with an aqueous solution of 75% isopropyl alcohol, or a mild detergent.

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 11/4 in, 5 amp, 250 volt; Type T (time delay) fuses.

Turn off power and remove power cord from power module. Use a straight blade screwdriver 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 (Underside of Instrument)

1. Disconnect from mains power supply.
2. Remove screw and swing cover to the side.
3. Slide battery out from under clip.
4. 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.

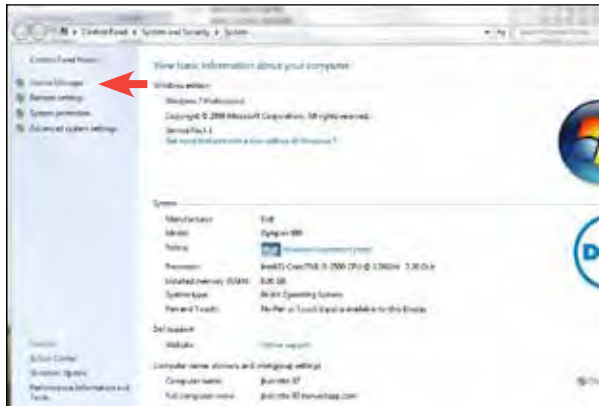
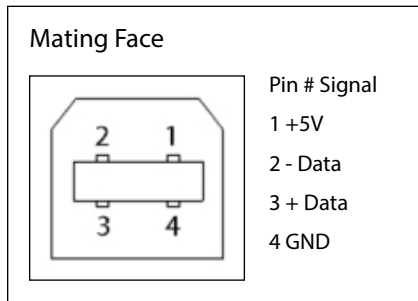


Upgrading ECM 830 Series Software

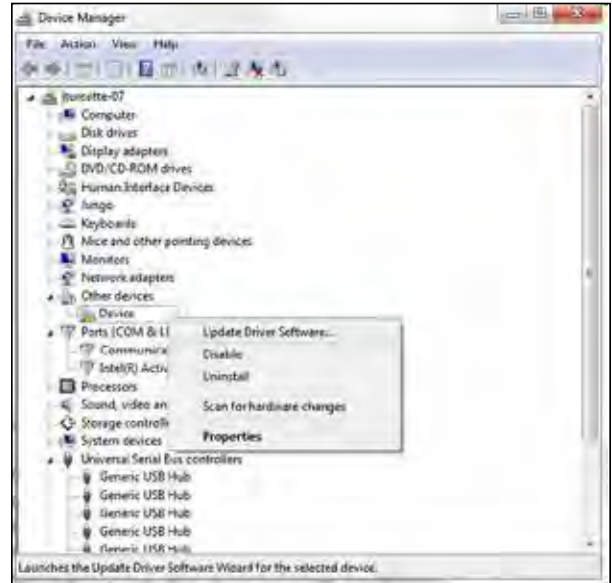
USB Virtual CommPort Driver Installation

When you connect the ECM 830 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 ECM 830.

1. Connect the ECM 830 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.



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**.



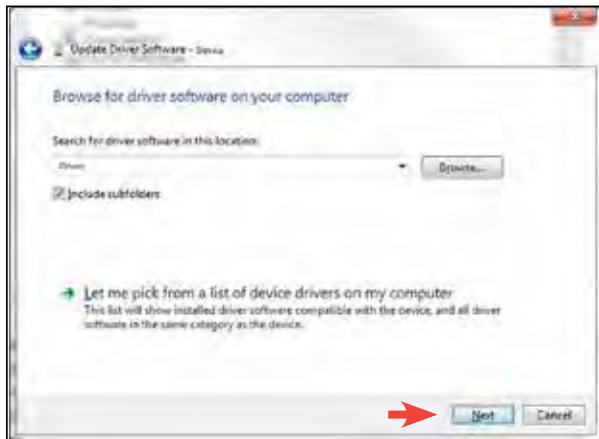
3. Select **Browse my computer for driver software**.



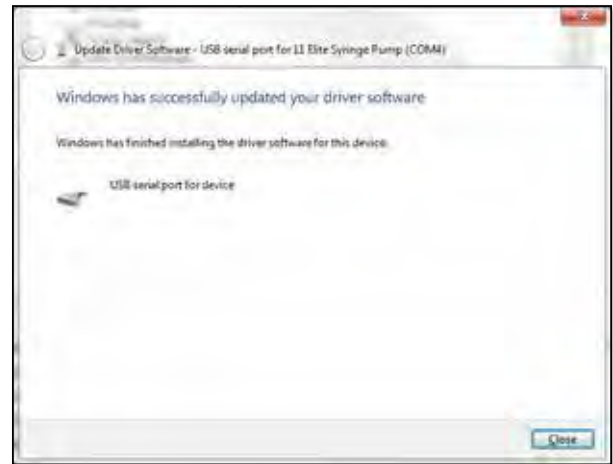
Upgrading ECM 830 Series Software

USB Virtual CommPort Driver Installation (continued)

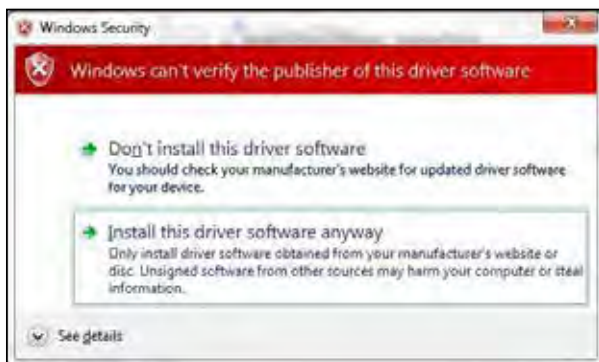
4. Download the software from BTX website www.btxonline.com. Click on the **Technical Resources** drop-down menu and select **Downloads**. Download and install file named **Firmware Updater**.



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



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



Upgrading ECM 830 Series Software

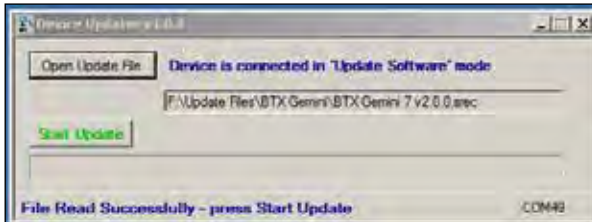
Firmware Updater Program

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

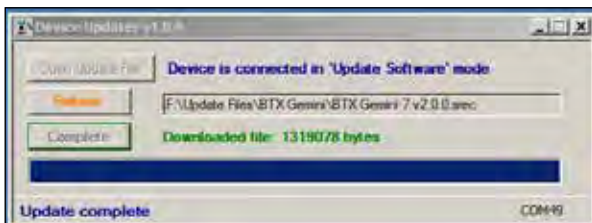
1. Upload the latest software versions to your desktop (format is filename.srec). There is one .srec file that needs to be updated: 'APP BTX_Gemini_upgrade x.x.x'.
2. Disconnect all I/O devices and then connect the ECM 830 generator to the PC using a USB cable.
3. On the setup screen of the ECM 830 generator, press the **Upgrade Software** icon twice to enter **Boot Loader Mode**.

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

4. Download the software from the BTX website www.btxonline.com. Click on the **Technical Resources** drop-down menu and select **Downloads**. Download and install file named **Firmware Updater**.



5. Click Open Update File and browse to the BTX Gemini 7 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 ECM 830 generator from the power switch in the back of the unit.

Error Messages & Troubleshooting

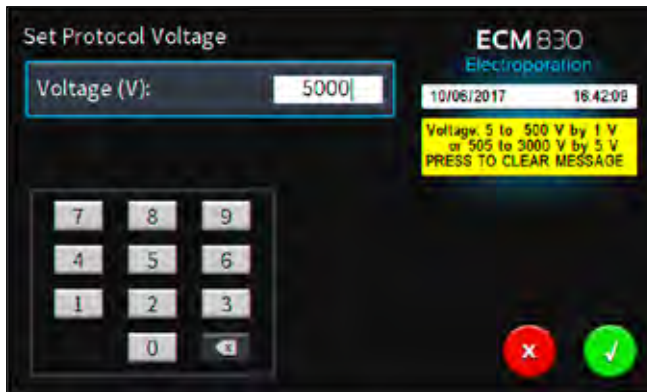
Display Error Message	Error Description	User Troubleshooting
Power Loss Error	Power loss during pulsing.	Power lost while protocol was running.
PFC Over Temp	PFC module over temperature.	Hardware error. Please contact the manufacturer.
Interlock Open	Electrode interlock open.	Connect electrode connectors to the front of the generator and make sure the cuvette safety dome cover is closed.
IGBT Switch Fault	IGBT switches fault.	Hardware error. Please contact the manufacturer.
Charge Voltage Max Error	Charging voltage above the expected voltage.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Charge Current Max Error	Charging current above the expected current.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
LV Cap Bank Max	Low voltage capacitor bank voltage above expected value.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
LV Cap Bank Min	Low voltage capacitor bank voltage below expected value.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
HV Cap Bank Max	High voltage capacitor bank voltage above expected value.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
HV Cap Bank Min	High voltage capacitor bank voltage below expected value.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Charge Time Max	Charging capacitor bank to selected voltage exceeded max time estimated.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Pulse Droop Error	Pulse voltage droop exceeded estimated droop.	A) Increase the sample resistance. This can be achieved by: <ol style="list-style-type: none"> 1. Increasing the gap size. 2. Reducing the volume. 3. Using a less conductive buffer. B) Reduce the pulse duration.
Pulse Voltage Overshoot	Pulse voltage monitor (p-vmon) pulse overshoot.	Hardware error. Please contact the manufacturer.
Pulse Voltage Mon too Low	Pulse voltage monitor (p-vmon) below min voltage.	Hardware error. Please contact the manufacturer.
Pulse Voltage Mon too High	Pulse voltage monitor (p-vmon) above max voltage.	Hardware error. Please contact the manufacturer.
Pulse Current Mon too Low	Pulse current monitor (p-imon) below min current.	Hardware error. Please contact the manufacturer.
Pulse Current Mon too High	Pulse current monitor (p-imon) above max current.	Hardware error. Please contact the manufacturer.
Arc_Detected	Arc detected during pulsing.	Arc could happen due to the one or more of the following conditions: <ol style="list-style-type: none"> 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.
No_HB_from_GUI	Communication error.	Hardware error. Please contact the manufacturer
Sample Resistance Out Of Range	The measured sample load is out of range.	Increase the sample resistance. This can be achieved by: <ol style="list-style-type: none"> 1. Increasing the gap size. 2. Reducing the volume. 3. Replacing the buffer with less conductive type of buffer.
Over Current Pulse Abort	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: <ol style="list-style-type: none"> 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.
Idle V I Max	Voltage or current in idle state is higher than expected.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Charge Cap Bank A Max	Charge capacitor bank A voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Charge Cap Bank A Min	Charge capacitor bank A voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Charge Cap Bank B Max	Charge capacitor bank B voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Charge Cap Bank B Min	Charge capacitor bank B voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Charge Cap Bank C Max	Charge capacitor bank C voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Charge Cap Bank C Min	Charge capacitor bank C voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Dump Time Max	The capacitor bank did not fully discharge in the estimated time.	Turn off the unit for a few minutes then turn back on. Rerun the protocol. If the issue recurs, contact the manufacturer.
Invalid Protocol	The generator was requested to run an invalid protocol.	Verify that the used protocol is within the allowed specifications of the generator.
System Processing (Busy)	The control command is invalid or was issued while the generator was busy.	Allow more time between events
TC_Error	The measured rc time while delivering an exponential decay pulse is invalid.	Hardware error. Please contact the manufacturer.
Pulse Duration Error	The pulse duration did not meet the expected duration.	Hardware error. Please contact the manufacturer.
Unit Type Sel Error	The unit hardware and software do not match the correct type.	Hardware error. Please contact the manufacturer.
Error: 0X#	Multiple errors.	Multiple error. Please contact the manufacturer.

Troubleshooting

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

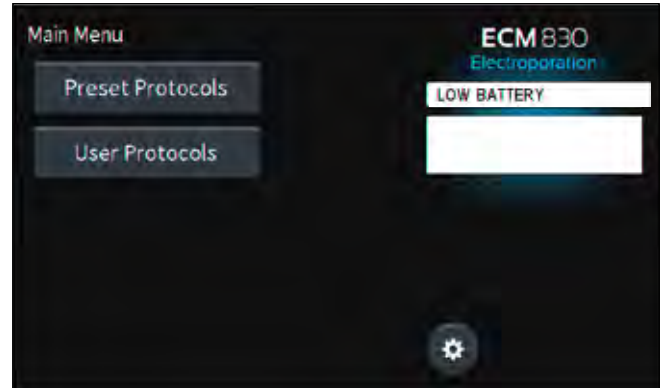
Out of Range

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



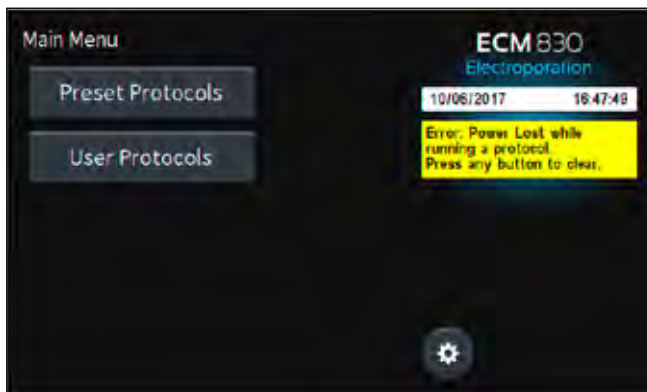
Low Battery Indication

Time/Date area will flash between Date and Time and LOW BATTERY message will display.



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 on-screen message is displayed to indicate the interruption.



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. Are the voltage, chamber gap, pulse length (time constant), pulse number and other instrument settings correct? If so, reduce voltage, pulse length, or number of pulses and reoptimize 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%.

General Optimization Guide for Electroporation

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 biomembrane. 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, and 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 when the voltage pulse is applied to the cells and medium is 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:

$$\text{TMV} \approx E r \cos \alpha$$

where:

E = electric field intensity in volts/cm

r = the cell radius in cm

α = 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 μm 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).

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 continues until the pores close, which can take milliseconds to minutes.

Optimization of the electroporation process involves several factors. Choosing the waveform, 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.

Waveforms

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, maintain this level during the duration of the set pulse length and quickly turn off. Square waves yields higher efficiencies and viabilities in mammalian cells. Square wave electroporation in in vivo and ex vivo tissues, embryos, 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. As 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 bacteria, 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 Type 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 4 mm gap cuvette with 500 V would provide a field strength of 1.25 kV/cm. If instead of a 4 mm gap cuvette, a 2 mm gap cuvette was used, the voltage would have to be reduced by half or to 250 V in order to maintain the same field strength of 1.25 kV/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 1 mm gap cuvette the calculation would be: 1.25 kV x 0.1 cm = 0.125 kV or 125 V.

Example: A field strength of 1.25 kV/cm

4 mm gap cuvette = 500 V

2 mm gap cuvette = 250 V

1 mm gap cuvette = 125 V

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 lower 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 ranges from microseconds to milliseconds. Adjusting this parameter is dependent on the pulse waveform. 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 one-third the original set voltage. This time constant is modified by adjusting the resistance and capacitance (RC) values in an exponential decay waveform. 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 and 100 volts with pulse lengths ranging 30 to 50 ms 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 effect 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 a 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, Lowering the volume will increase the resistance and decrease the arc potential.

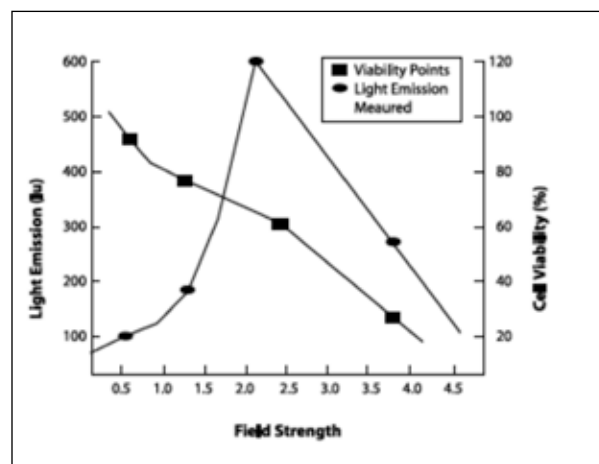
BTX offers BTXpress High Performance Electroporation Solution, a low conductance buffer that achieves higher transfection efficiencies with minimal cell toxicity. The BTXpress buffer is a single buffer developed to facilitate high efficiency gene delivery into mammalian cells.

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 – 20 µg/ml for most cell types; however in some instances increasing the DNA concentration as high as 50 µg/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 diH₂O, dialysis, or ethanol precipitation can significantly improve transformation efficiencies and reduce the potential for arcing.

Protocol Optimization In Vitro



Choose the optimal field strength based on the best conditions observed when plotting viability versus expression at different field strengths.

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 and yield high transfection efficiencies and great cell survival rates.

In Vivo, In Utero, In Ovo

Square wave systems 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 zebrafish.

Electroporation Buffers

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

The following is a list of the most commonly used buffers and media:

BTXpress — 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 — 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 — 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 — 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 — 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-MEM 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 — suitable for a diverse spectrum of mammalian cell types. Various formulations available with either Hank’s or Earle’s salts.

DMEM — 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 — a composition of cytokines for the highly efficient and reproducible expansion of human multipotent mesenchymal stromal cells (MSCs).

Water and 10% glycerol — typically used for bacteria

Cytoporation Media T — 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 — 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.

Ordering Information

Order No.	Description	Qty.
Electroporation Systems		
45-2052	ECM 830, System with Safety Dome Includes ECM 830 Generator, Safety Dome, Cuvettes 1 mm, 2 mm, 4 mm pkg. of 30 (10 each) and Cuvette Rack 660	1 ea
45-0661	ECM 830, System with Safety Stand ECM 830 Generator, 630B Safety Stand, Cuvettes 1 mm, 2 mm, 4 mm pkg. of 30 (10 each) and Cuvette Rack 660	1 ea
45-0662	ECM 830 Generator only	1 ea
45-0663	ECM 830, High Throughput System, 25-well, with HT-100, Includes ECM 830 Generator, 25-Well Plates (2 mm gap, 6X), Plate Seals, HT-100 Plate Handler and a plate adaptor	1 ea
45-0664	ECM 830, High Throughput System, 25-well, with HT-200, Includes ECM 830 Generator, 25-Well Plates (2 mm gap, 6X), Plate Seals, HT-200 Plate Handler and a plate adaptor	1 ea
45-0665	ECM 830, High Throughput System, 96-well, with HT-100, Includes ECM 830 Generator, 96-Well Plates (2 mm gap, 2X), Plate Seals, HT-100 Plate Handler and a plate adaptor	1 ea
45-0666	ECM 830, High Throughput System, 96-well, with HT-200, Includes ECM 830 Generator, 96-Well Plates (2 mm gap, 2X), Plate Seals, HT-200 Plate Handler and a plate adaptor	1 ea
45-0667	ECM 830, System with Monitoring Includes ECM 830 Generator, 630B Safety Stand, Cuvettes 1 mm, 2 mm, 4 mm pkg. of 30 (10 each), Cuvette Rack 660, Enhancer 3000 Probe, Enhancer Interface Box, Oscilloscope and Cables	1 ea
45-0668	ECM 830, High Throughput System, 96-well, with Monitoring, Includes ECM 830 Generator, 630B Safety Stand, Cuvettes 1 mm, 2 mm, 4 mm pkg. of 30 (10 each), Cuvette Rack 660, Enhancer 3000 Probe, Enhancer Interface Box, Oscilloscope, Cables, 25-Well Plates (2 mm gap, 6X), HT-200 Plate Handler and a plate adaptor	1 ea
Cuvettes		
45-0134	Cuvette Plus, 1 mm gap, 90 µl, Sterile Pkg/10, Gray	1 pkg
45-0124	Cuvette Plus, 1 mm gap, 90 µl, Sterile Pkg/50, Gray	1 pkg
45-0140	Cuvette Plus, 1 mm gap, 90 µl, Sterile 24 Pkg/100 each, Gray	24 pkg
45-0135	Cuvette Plus, 2 mm gap, 400 µl, Sterile Pkg/10, Blue	1 pkg
45-0125	Cuvette Plus, 2 mm gap, 400 µl, Sterile Pkg/50, Blue	1 pkg
45-0141	Cuvette Plus, 2 mm gap, 400 µl, Sterile 24 Pkg/100 each, Blue	24 pkg
45-0136	Cuvette Plus, 4 mm gap, 800 µl, Sterile Pkg/10, Yellow	1 pkg
45-0126	Cuvette Plus, 4 mm gap, 800 µl, Sterile Pkg/50, Yellow	1 pkg
45-0142	Cuvette Plus, 4 mm gap, 800 µl, Sterile 24 Pkg/100 each, Yellow	24 pkg

Order No.	Description	Qty.
Reagents		
47-0002	BTXpress Cytoporation Low Conductivity Medium T, 500 ml volume	1 bottle
47-0003	BTXpress Cytoporation Low Conductivity Medium T4, 500 ml volume	1 bottle
45-0801	BTXpress Solution, 2.0 ml Bottle, For up to 20 Reactions	1 bottle
45-0802	BTXpress Solution, 5.0 ml Bottle, For up to 50 Reactions	1 bottle
45-0805	BTXpress Solution, 10.0 ml Bottle, For up to 100 Reactions	1 bottle
Accessories		
45-0495	Oocyte Electrode, Platinum Plated, 10 mm, 1 mm gap (Electrode only)	1 ea
45-0496	Oocyte Electrode Kit, Platinum Plated, 10 mm, 1 mm gap, (with cables)	1 kit
45-0110	Flatpack Chambers, 0.56 mm gap, 80 µl volume, pkg. of 50 (Requires Safety Stand 45-0207 or 45-0208)	Pkg of 50
45-0109	Flatpack Chambers, 1.83 mm gap, 1.5 ml volume, pkg. of 50 (Requires Safety Stand 45-0207 or 45-0208)	Pkg of 50
47-0206	Flatpack Chambers, 4 mm gap, 10 ml volume, pkg. of 10 (Requires Safety Stand 45-0208)	Pkg of 10
45-2021	Safety Dome for cuvettes	1 ea
45-0207	Safety Stand for cuvettes (also accepts small volume Flatpacks 45-0110 and 45-0109)	1 ea
47-0208	Safety Stand for Flatpack (accepts cuvettes and all sizes of Flatpack)	1 ea
45-0400	HT-100 Plate Handler, Manual Track Switching	1 ea
45-0401	HT-200 Plate Handler, Auto-Sense Track Switching	1 ea
45-0465	HT 25 adapter for Plate Handlers	1 ea
45-0468	Replacement pins, pkg. of 25	1 pkg
45-0469	Replacement pins, pkg. of 100	1 pkg
5012-017	Pliers for Changing Pins between 96-Well and 25-Well formats	1 ea
45-0466	25-Well Disposable Electroporation Plate, 2 mm gap, 125 µl, coated, 1 plate	1 ea
45-0466-M	25-Well Disposable Electroporation Plate, 2 mm gap, 125 µl, 1 plate	1 ea
45-0467	25-Well Disposable Electroporation Plate, 2 mm gap, 125 µl, pkg. of 6	1 pkg
45-0462	25-Well Disposable Electroporation Plate, 4 mm gap, 250 µl, 1 plate	1 ea
45-0463	25-Well Disposable Electroporation Plate, 4 mm gap, 250 µl, pkg. of 6	1 pkg
45-0450	96-Well Disposable Electroporation Plate, 2 mm gap, 125 µl, Coated, 1 plate	1 ea
45-0450-M	96-Well Disposable Electroporation Plate, 2 mm gap, 125 µl, 1 plate	1 ea
45-0452	96-Well Disposable Electroporation Plates, 4 mm gap, 250 µl, 1 plate	1 ea
45-00012	25-Well Plate Seal (1)	1 ea
45-00015	96-Well Plate Seal (1)	1 ea
45-2030	Gemini X2/ECM 830/ECM 630 Foot Pedal	1 ea
45-0122	Genepaddles, 3 x 5 mm (requires 45-0203 holder and 45-0216 cables sold separately)	1 ea

Ordering Information

Order No.	Description	Qty.
45-0169	Genepaddles Kit, 3 x 5 mm (includes 45-0203 holder and 45-0216 cables)	1 kit
45-0123	Genepaddles, 5 x 7 mm (requires 45-0203 holder and 45-0216 cables sold separately)	1 ea
45-0170	Genepaddles Kit, 5 x 7 mm (includes 45-0203 holder and 45-0216 cables)	1 kit
45-0203	Genetrode/Genepaddle Holder	1 ea
45-0117	Genetrodes, 1 mm L-Shape (GOLD TIP) (requires 45-0203 holder and 45-0216 cables sold separately)	1 ea
45-0164	Genetrodes, 1 mm L-Shape (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 kit
45-0116	Genetrodes, 3 mm L-Shape (GOLD TIP) (requires 45-0203 holder and 45-0216 cables sold separately)	1 ea
45-0163	Genetrodes, 3 mm L-Shape (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 kit
45-0115	Genetrodes, 5 mm L-Shape (GOLD TIP) (requires 45-0203 holder and 45-0216 cables sold separately)	1 ea
45-0162	Genetrodes Kit, 5 mm L-Shape (GOLD TIP) with 45-203 holder and 45-0216 cables	1 kit
45-0113	Genetrodes, 5 mm Straight (GOLD TIP) (requires 45-0203 holder and 45-0216 cables sold separately)	1 ea
45-0160	Genetrodes Kit, 5 mm Straight (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 kit
45-0114	Genetrodes, 10 mm Straight (GOLD TIP) (requires 45-0203 holder and 45-0216 cables sold separately)	1 ea
45-0161	Genetrodes Kit, 10 mm Straight (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 kit
45-0509	Needle L-Shaped Platinum Electrode, 3mm (requires 45-0508 cables sold separately)	1 ea
45-0510	Needle L-Shaped Platinum Electrode Kit, 3 mm, with 45-0508 cables	1 kit
45-0530	Adherent Cell Electrode, 5 mm gap, For 12 well dish adherent electroporation (requires 45-0204 cables sold separately)	1 ea
45-0531	Adherent Cell Electrode, 5 mm gap, For 12 well dish adherent electroporation with 45-0204 cables	1 kit
45-0130	Petri Pulsar, 2 mm gap, For 35 mm dish adherent electroporation	1 ea
45-0100	Petri Dish Electrode, 2 mm gap, For 10 cm dish adherent electroporation	1 ea
45-0504	Petri Dish Platinum Electrode Chamber for Tissues, 5 mm gap (requires 45-0216 cables sold separately)	1 ea
45-0505	Petri Dish Platinum Electrode Chamber Kit for Tissues, 5 mm gap, with 45-0216 cables	1 kit
45-0506	Petri Dish Platinum Electrode Chamber for Tissues, 15 mm gap (requires 45-0216 cables sold separately)	1 ea
45-0513	Petri Dish Tissue Chamber Kit for Tissues, 15 mm gap, with 45-0216 cables	1 kit
45-0491	Petri Dish Platinum Electrode Chamber for Tissue Slices, 7 mm, negative	1 ea
45-0492	Platinum Electrode Wand for Tissue Slices, 7 mm, positive	1 ea

Order No.	Description	Qty.
45-0490	Petri Dish Platinum Electrode for Tissue Slices Chamber Kit, 7 mm, with 45-0491 chamber, 45-0492 wand 7 mm, 45-0503 cables, 45-0204 cables	1 kit
45-0501	Petri Dish Platinum Electrode Chamber for Tissue Slices, 10 mm, negative	1 ea
45-0502	Platinum Electrode Wand for Tissue Slices, 10 mm, positive	1 ea
45-0500	Petri Dish Platinum Electrode for Tissue Slices Chamber Kit, 10 mm, with 45-0501 chamber, 45-0492 wand 7 mm, 45-0503 cables, 45-0204 cables	1 kit
45-0490	Petri Dish Platinum Electrode for Tissue Slices Chamber Kit, 10 mm, with 45-0491 chamber, 45-0502 wand 7 mm, 45-0503 cables, 45-0204 cables	1 kit
45-0524	Platinum Tweezeretrode, 1 mm Flat (requires 45-0204 cables sold separately)	1 ea
45-0525	Platinum Tweezeretrode Kit, 1 mm Flat (includes 45-0204 cables)	1 kit
45-0486	Platinum Tweezeretrode Kit, 1 mm Diameter (includes 45-0204 cables)	1 kit
45-0487	Platinum Tweezeretrode Kit, 3 mm Diameter (includes 45-0204 cable)	1 kit
45-0489	Platinum Tweezeretrode Kit, 5 mm Diameter (includes 45-0204 cables)	1 kit
45-0118	Stainless Tweezeretrode Electrode, 7 mm Diameter (requires 45-0204 cables sold separately)	1 ea
45-0165	Stainless Tweezeretrode Kit, 7 mm (includes 45-0204 cable)	1 kit
45-0488	Platinum Tweezeretrode, 7 mm Diameter (includes 45-0204 cables)	1 kit
45-0119	Stainless Tweezeretrode Electrode, 10 mm Diameter	1 ea
45-0166	Stainless Tweezeretrode Kit, 10 mm (includes 45-0204 cable)	1 kit
45-0493	Triple Electrode Tweezeretrode Kit, 3 mm (includes connection box)	1 kit
45-0494	Triple Electrode Tweezeretrode Kit, 5 mm (includes connection box)	1 kit
45-0121	2-Needle Array Electrode Tips, 5 mm gap, Pkg. of 6	1 pkg
45-0206	2-Needle Array Handle, for 5mm gap tips	1 ea
45-0168	2-Needle Array Kit, 5 mm, Pkg. of 6, with Handle	1 kit
45-0120	2-Needle Array Electrode Tips, 10 mm gap, Pkg. of 6	1 pkg
45-0205	2-Needle Array Handle, for 10 mm gap tips	1 ea
45-0167	2-Needle Array Kit, 10 mm, Pkg. of 6, with Handle	1 kit
45-0101	Caliper Electrode , 1.0 cm X 1.0 cm, Brass, Transdermal Delivery Applications	1 ea
45-0102	Caliper Electrode, 2.0 cm x 2.0 cm with extra 1.5 cm x 1.5 cm electrodes included, Stainless Steel, Electrochemotherapy Applications	1 ea

Ordering Information

Order No.	Description	Qty.
Cables & Adapters		
45-0087	Adaptor Set, Micrograbber to Banana Plug Cables	1 ea
45-0216	Micrograbber to Banana Plug Connection Cables, Red and Black, 10 ft (for use with microslides, genetrodes and tissue petri dish)	1 ea
45-0503	Micro-Grabber Cable for Tissue Slice Chamber and Oocyte electrode	1 ea
45-0204	Adapter Banana Plug Cables, Red and Black (for use with Tweezertrodes and L-Shaped Gentrodes Electrodes)	1 ea
45-0088	Female/Female Adapter Set for Banana Plug Cables	1 ea
45-0217	Banana to Banana Cables, Red and Black, 10 ft	1 ea