

ECM 2001

Electrofusion & Electroporation System

USER'S MANUAL



BTX[®]

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

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A Message from BTX

Thank you for investing in the ECM 2001+ 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: 800.272.2775

International Callers: 508.893.8999

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



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Warranty Information

RESEARCH ONLY

BTX

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Warranty

BTX warrants the 2001+ Electrofusion & 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 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 BTX 2001+ 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 800.547.6766 (US Only) or 508.893.8999 (E-mail: repairs-returns@harvardbioscience.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 BTX 2001+ Electrofusion and 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) and safety.

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 a line cord that is certified for country of use. The Operating voltage range 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 is is difficult to manage the connection and disconnection of devices.

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 800.272.2775, or 508.893.8999.

Caution Notice

The BTX 2001+ Electrofusion & 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

Product Overview

The BTX ECM 2001+ is a multifunctional electrofusion and electroporation square wave generator.

Fast, efficient cell fusion in hybridoma production, hybrid cell formation and nuclear transfer applications are facilitated by the combination of AC and DC wave pulses. Fusion is achieved by the generation of an AC current waveform that generates a benign dielectrophoretic alignment of cells. With a 30 ms switchover time from AC to DC, efficient fusion takes place. After fusion, the AC is reapplied maintaining the cell compression for the rounding off process resulting in a higher number of hybrids.

Square waveform electroporation is an efficient non-viral method for transfecting genes and other molecules into mammalian cell lines. This technology is commonly used to study gene targeting, function and to understand protein regulation. Electroporation is a standard method used to transfect mammalian cell lines to express recombinant human proteins which are used for therapeutic purposes. Gene delivery by this method is typically used to create a transient transfection in order to study protein expression or to temporarily knockout or “silence” these genes using siRNA. This is used to study gene targeting and function. Alternatively, stable transfection integrates a gene into the genome of the cell for long term expression of protein. The use of the ECM 2001+ offers the control needed to adjust electrical settings for optimization of parameters. This system is powerful enough to yield high transfection efficiencies for cell lines and difficult to transfect cell types including stem cells and primary cells. The gentle square wave pulse also allows for high cell viability of these cell types.

ECM 2001+ features include:

- **AC waveform of 0.2 – 2.0 MHz**
- **Electroporation capabilities**
- **A wide range of voltages from 5 V to 3000 V**
- **Finer voltage discrimination**
- **Capable of operating at low impedance loads**



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

Unpacking the System

The shipping carton in which your ECM 2001+ 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 2001+ 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 100–240 VAC, 50/60 Hz.

The power requirements are 350 watts. The unit is shipped with a standard 3-prong plug for North America customers. An additional region-specific power cord is provided for customers outside of North America.

Installation

Once you have determined that the components of the system have not sustained any obvious damage in shipment, proceed with the installation.

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.

Allow unit to come to stable room temperature. Pull the plastic tab from the battery holder at the bottom of the unit.

Connect the mains/power cord into the back panel.

Power up the system by pushing the rocker switch located on the back panel. The display will show a progress bar as the system is initializing. Once the software initializes, the splash screen will appear, showing the BTX logo.

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.



Cancel

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



Back

Used to go back one screen.



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.



Pre-Pulse Resistance Measurement

Used to measure the resistance of the sample prior to delivering the protocol.



Run Protocol

Used to deliver the pulse protocol to the sample.



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.



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.



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.



Add a Step

Used to add an additional step to a User Protocol.



Delete a Step

Used to remove the highlighted step from a User Protocol.



Copy a Step

Used to copy the step that is currently highlighted in a User Protocol.



Move Step Position One Up

Used to move the highlighted step in a User protocol one position up in the sequence of steps.



Move Step Position One Down

Used to move the highlighted step in a User protocol one position down in the sequence of steps.



Delete User Protocol



Accept Value Entered on Numeric Keypad



Accept entry on alphabetic keypad



Backspace, Delete One Character on Numeric and Alphabetic Keypad



Cancel entry on numeric and alphabetic keypad

Software Setup

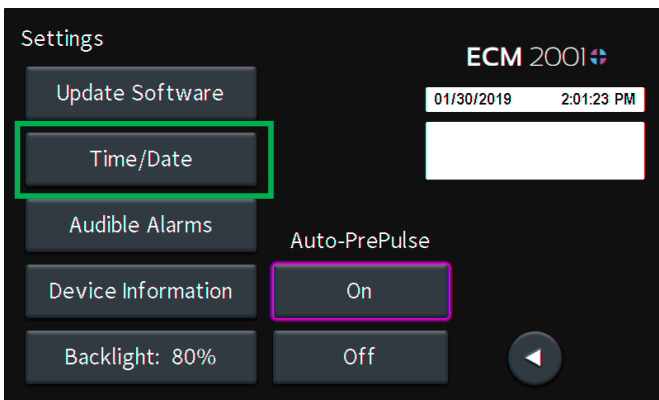
Setting Time/Date

After initializing, the splash screen will appear.

Tap the **Gear** icon.



Tap **Date/Time** icon.

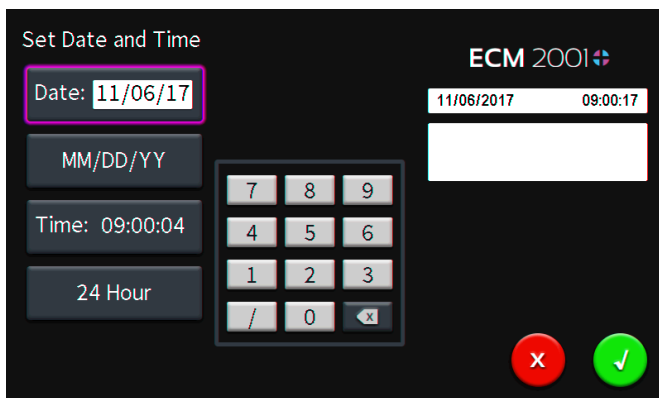


Tap **Date** icon and use keypad to enter the date.

Tap **Time** icon and use keypad to enter the time.

Tap **Green Check** icon to accept changes and to return the **Settings Screen**.

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

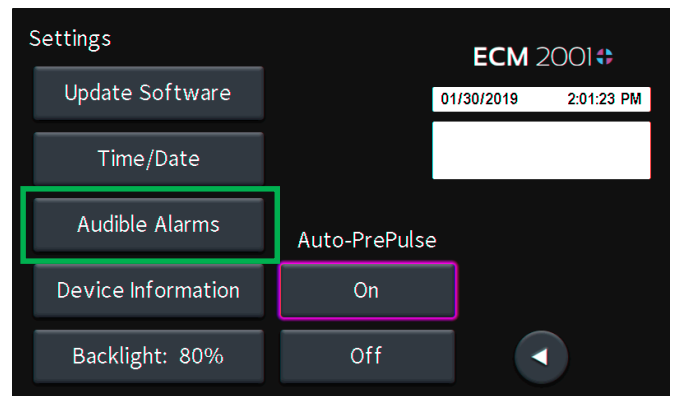


Setting Audible Alarm Preferences

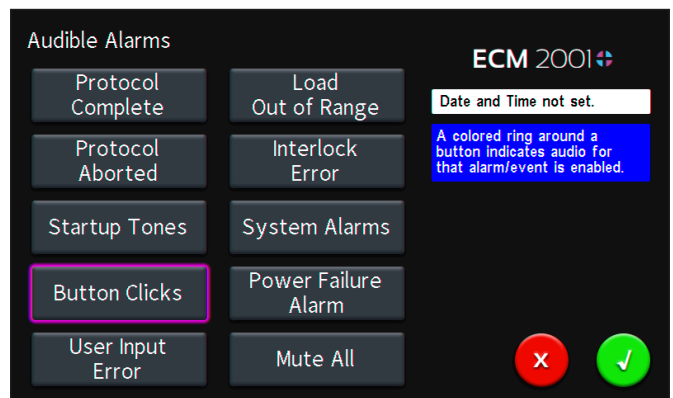
From the splash screen, tap the **Gear** icon.



Tap **Audible Alarms** icon.



Select preferred **Audible Alarms** by tapping icons to activate or deactivate.



NOTE: The **Mute All** icon allows you to toggle off all selections or restore previous selections if tapped again. Activated alarms are indicated by a colored ring around the activated alarm button.

Tap **Green Check** icon to accept changes and to return the **Settings Screen**.

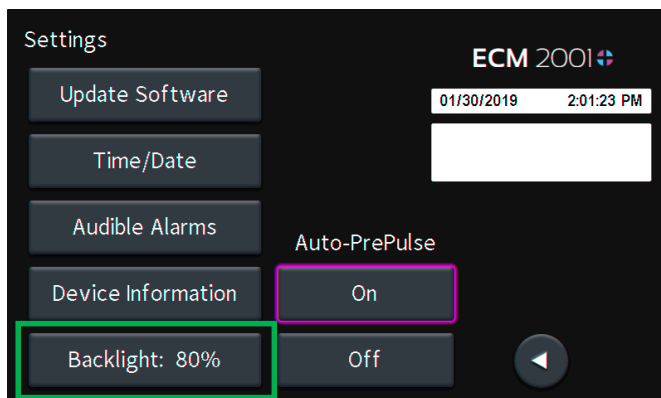
Software Setup

Setting Backlight Preferences

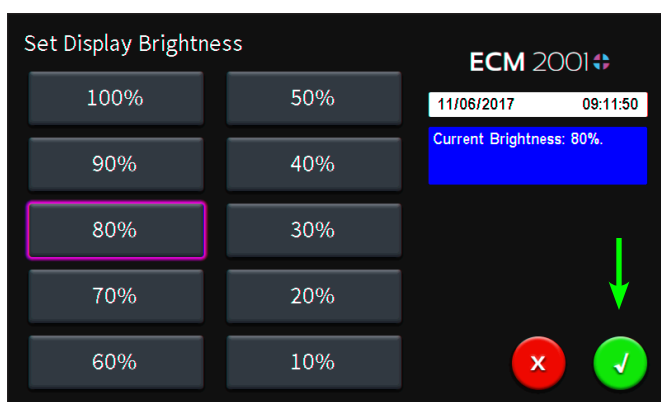
From the splash screen, tap the **Gear** icon.



Tap **Backlight** icon.



Select preferred brightness by tapping the icon indicating % brightness.



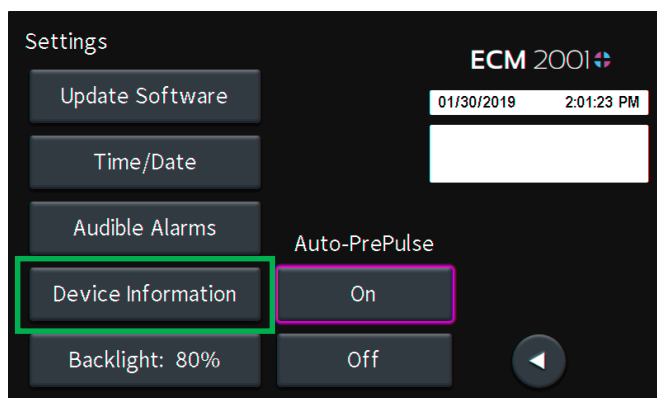
Tap **Green Check Icon** to accept changes and to return the **Settings Screen**.

Displaying Device Information

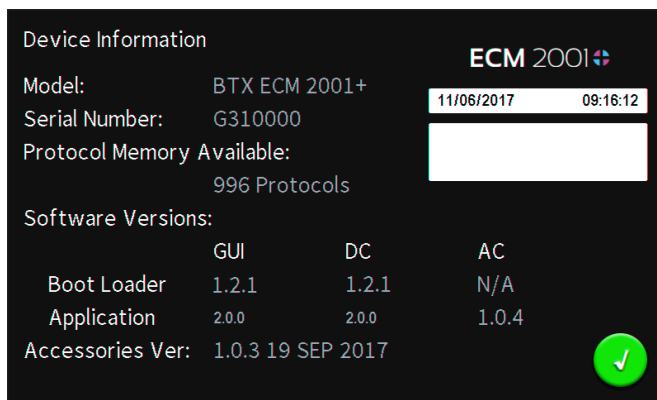
From the splash screen, tap the **Gear** icon.



Tap **Device Information** icon.



View device information.



Tap **Green Check Icon** to return the **Settings Screen**.

Software Setup

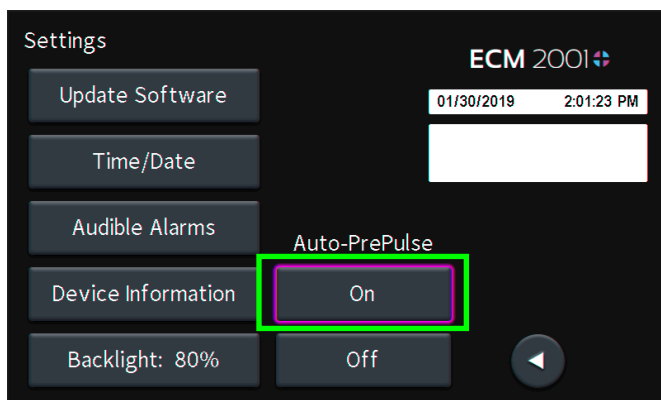
Toggling Auto-PrePulse Off/On

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."

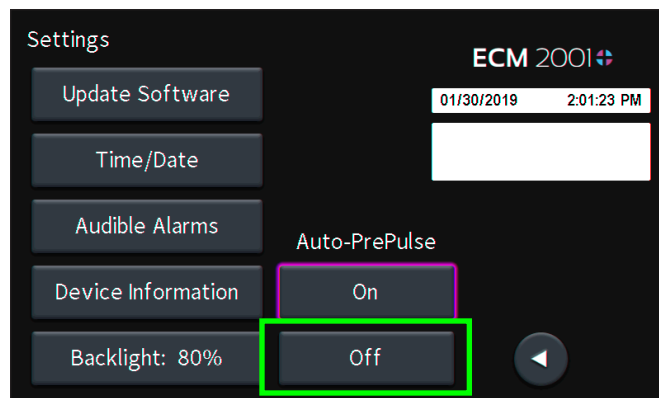
From the splash screen or the protocol run screen, tap the **Gear** icon to go to Settings.



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.

Software Setup

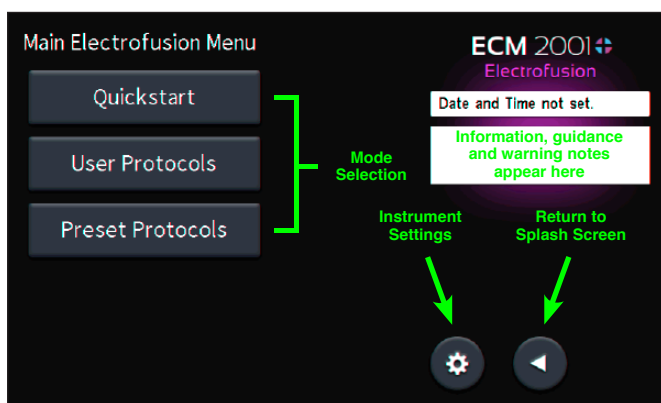
Electrofusion Screen Overview

After initializing, the splash screen will appear.

Tap the **Electrofusion** icon.



Electrofusion Main Menu



Once selecting 'Electrofusion' from the splash screen, all screens will have the heading '**Electrofusion**' in the upper right hand corner to indicate that you are in the Electrofusion function of the instrument. This will be changed to '**Electroporation**' when the Electroporation function has been selected from the splash screen.

Mode Selection

Select the desired electrofusion made by tapping one of the three displayed icons:

Quickstart	Standard 3-step protocol with guided input depending on the electrofusion accessory that has been selected. These protocols allow some flexibility and are suitable for most applications.
User Protocols	Custom protocols that are programmed by the user. Choose this option when more than 3-step protocols are needed. Previously saved custom user protocols can be found here.
Preset Protocols	These protocols are based on the most popular methods. These protocols are factory set and cannot be changed. Simply select protocol based on cell type and application.

Notification Box

This box, located just under the Date and Time banner, provides information on the status of a run, context-sensitive guidance for changing parameters that affect the run and warnings for when conditions and settings are outside the acceptable range for safe use. The following color scheme indicates the type of message and the actions (if any) required:

Information / Status Message

Provides information relevant to the current application or guidance for next steps if appropriate. During protocol programming, ranges of acceptable values will be displayed. There is no user input required for these messages.

Warning Message

This type of message indicates that a condition has arisen that requires user acknowledgment, but does not prevent operation of the system. Warning messages include a user instruction to verify that the protocol parameters are within the specifications for the selected accessory. Failure to take correct action in response to this message could lead to improper operation of the system or damage to the accessory. A Warning Message is also used to ask for user confirmation for certain actions, such as deleting a User Protocol. The user must acknowledge Warning Messages by tapping on the Notification Box to clear the message before further action is allowed.

Failure Message

This type of message indicates that there was a failure to complete an operation, and steps must be taken to correct this issue in order to complete the operation. The cause may be a user input error (such as attempting to set a parameter to an invalid value), failure of a protocol, or a system error (such as failure of a hardware component). The Notification Box will contain text and/or a fault code (refer to "Error Messages and Troubleshooting") to help the user diagnose the issue and recover if possible. The user must acknowledge failure messages by tapping on the Notification Box to clear the message before further action is allowed.

Instrument Settings

The gear icon allows you to navigate to the **Instrument Settings** from this screen. This sets generator settings for both **Electrofusion** and **Electroporation** functions of the unit.

Return to Splash Screen

This icon takes you back to Electrofusion or Electroporation Main Menu screen. To change from **Electrofusion** to **Electroporation** (or vice versa), press the **Back** icon once on this screen.

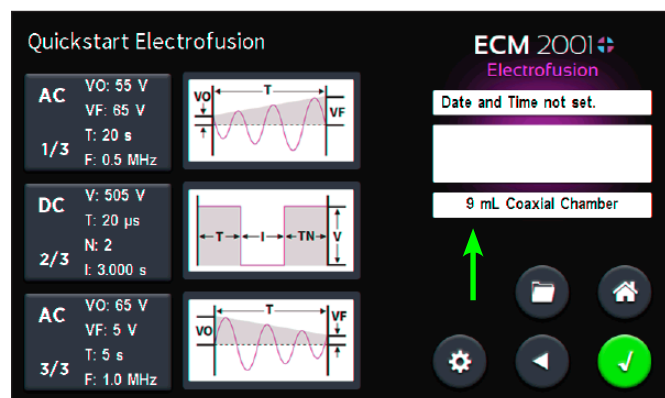
Software Setup

Electrofusion Quickstart

Setting AC Focusing Parameters

Tap the **Quickstart** icon.

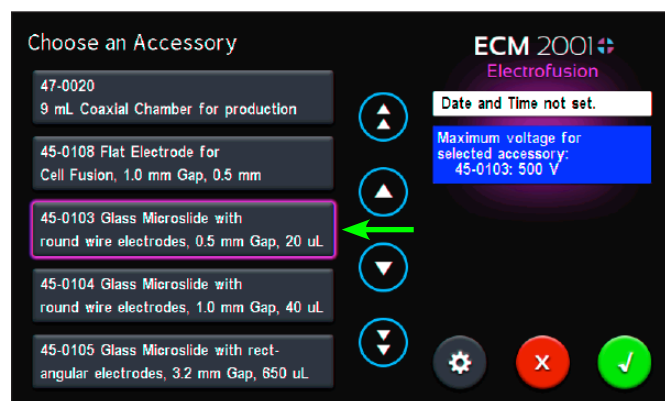
Confirm that the correct accessory is indicated in this window. If not, click on the window containing the accessory description to select a different one.



NOTE: It is important that the correct accessory is selected first. Selecting the accessory after programming protocol settings can result in errors that will prevent you from a successful run.

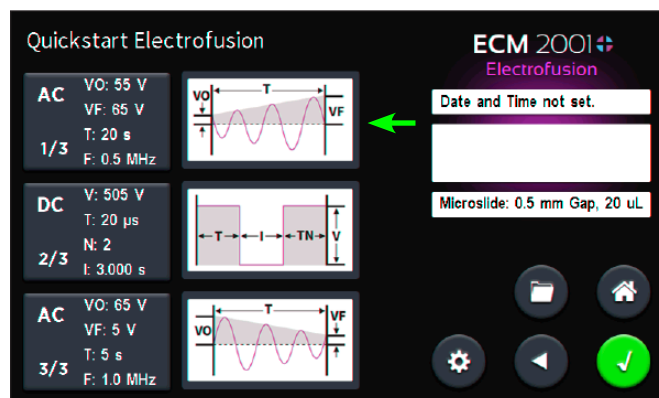
Use the up and down arrows to scroll through the list of compatible accessories.

Tap on the icon containing the name of the accessory you plan to use.



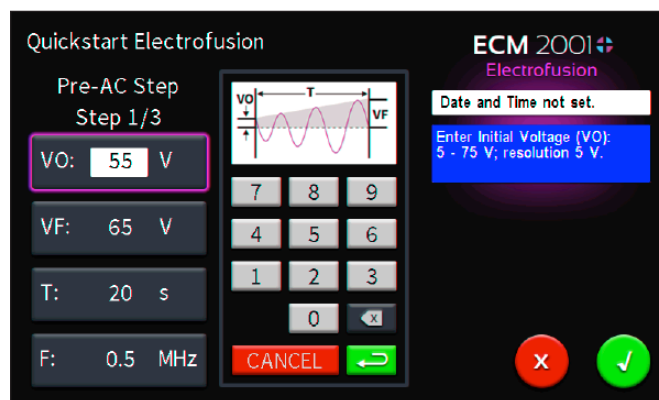
Tap the **Green Check** icon to accept accessory selection.

Tap the top icon (either on the graphic or on the box to the left of it) to set the cell alignment and compression step.



Use the central keypad to enter values for:

- **VO:** Starting AC voltage
- **VF:** Ending AC voltage
- **T:** Duration of AC cell focusing step in seconds
- **F:** AC Frequency



NOTE: The blue message box will provide you with appropriate ranges and resolutions for the accessory that you selected for this step.

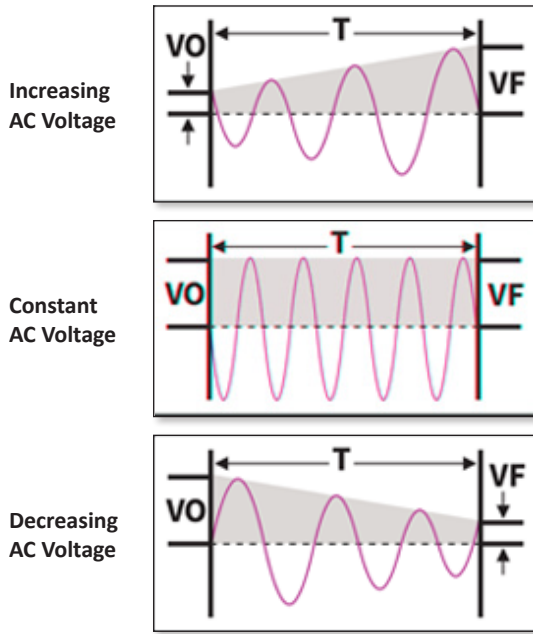
Tap the green **Arrow Key** to accept values or select another parameter by tapping that icon.

Software Setup

Electrofusion Quickstart (continued)

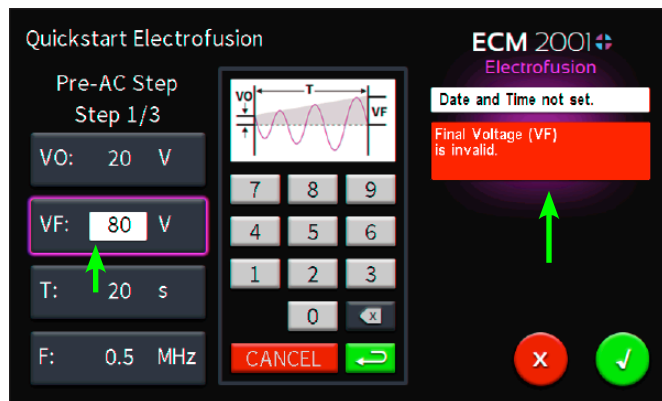
Setting AC Focusing Parameters (continued)

The graphical representation of the AC parameters set in this step may change due to your selections. These are not to scale:



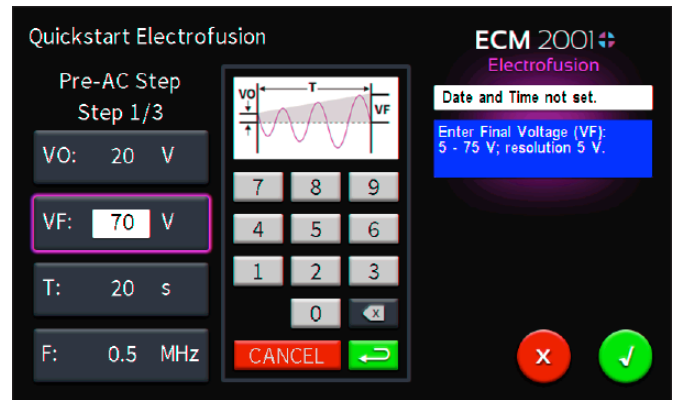
Tap the **Green Check** icon to accept all protocol values.

If any of the protocol values are invalid or out of range, you will see a red failure message that will indicate which values or value needs to be corrected.



In the above example, the final voltage (VF) has been set out of range.

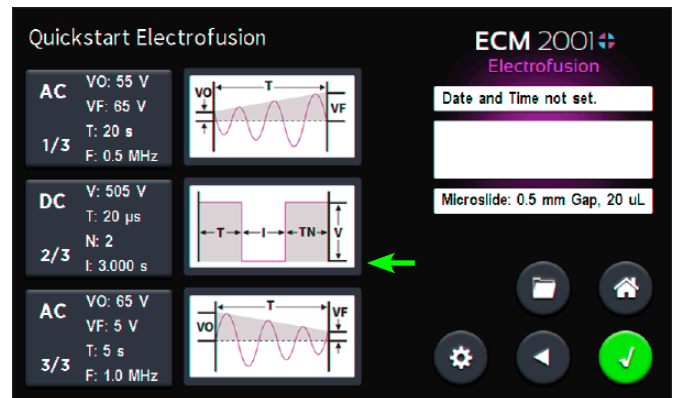
Tap the red message box to clear and then use the information in the blue information box to correct the value.



Tap the **Green Check** icon to accept all protocol values.

Setting DC Fusion Parameters

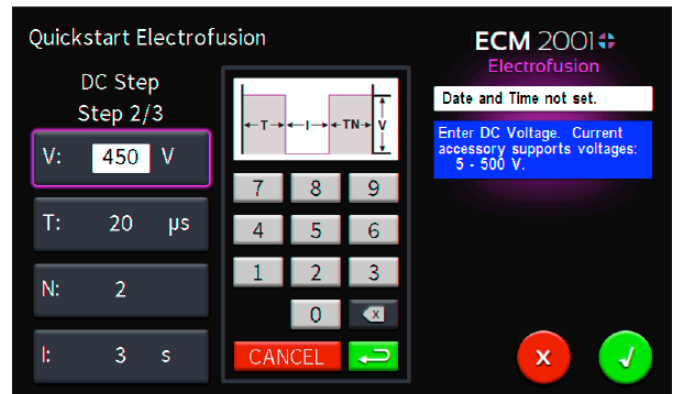
Tap the middle icon (either on the graphic or on the box to the left of it) to set the cell fusion step.



Use the central keypad to enter values for:

- **V:** DC Pulse voltage
- **T:** DC Pulse duration
- **N:** Number of pulses
- **I:** Pulse interval (time between multiple pulses)

Tapping the DC pulse duration units text (shown here as 'µs') toggles the DC pulse duration between microseconds (µs) and milliseconds (ms).



Tap the **Green Check** icon to accept values or select another parameter by tapping that icon.

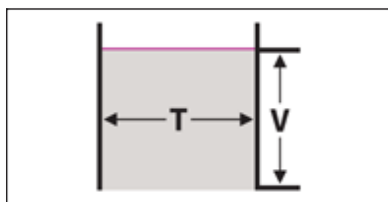
Software Setup

Electrofusion Quickstart (continued)

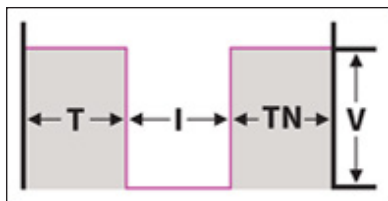
Setting DC Fusion Parameters (continued)

The graphical representation of the DC parameters set in this step may change due to your selections. These are not to scale:

1 DC Pulse



Multiple DC Pulses (2 to 10)



Setting Pulse Number to 0 will skip this step

No DC
(N = 0)

Setting Pulse Duration to 0 will skip this step

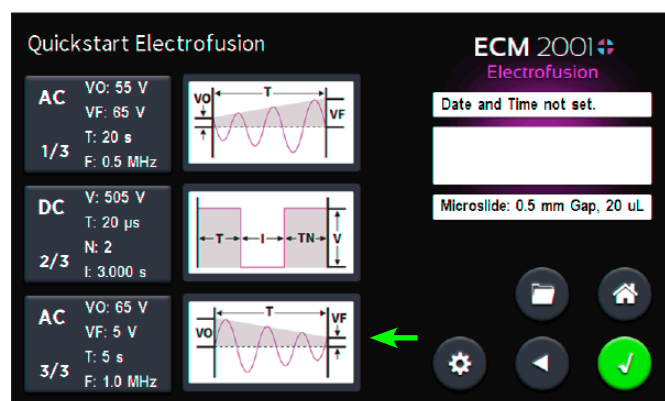
No DC
(T = 0)

NOTE: Skipping the DC fusion step can be useful when determining the best conditions for cell alignment and compression.

Tap the **Green Check** icon to accept all protocol values.

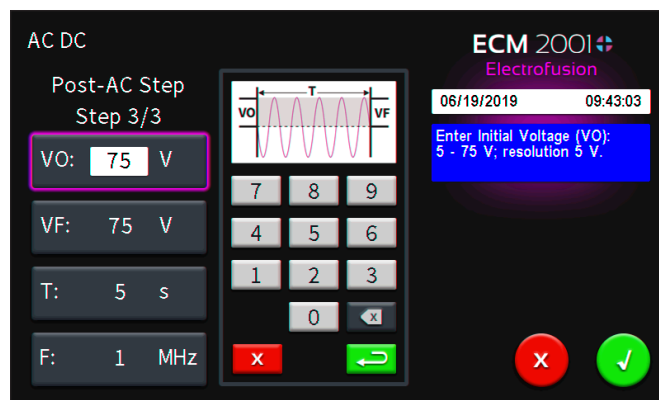
Setting Post-Fusion AC Parameters

Tap the bottom icon (either on the graphic or on the box to the left of it) to set the post-fusion AC step.



Use the central keypad to enter values for:

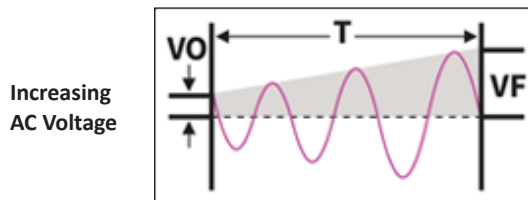
- **VO:** Starting AC voltage
- **VF:** Ending AC voltage
- **T:** Duration of AC cell stabilization step in seconds
- **F:** AC Frequency



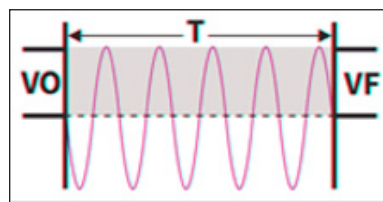
NOTE: The blue message box will provide you with appropriate ranges and resolutions for the accessory that you selected for this step.

Tap the **Green Check** icon to accept values or select another parameter by tapping that icon.

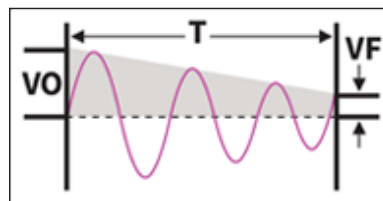
The graphical representation of the AC parameters set in this step may change due to your selections. These are not to scale:



Constant AC Voltage



Decreasing AC Voltage



Setting Time to 0 Seconds will skip this step

No AC
(T=0)

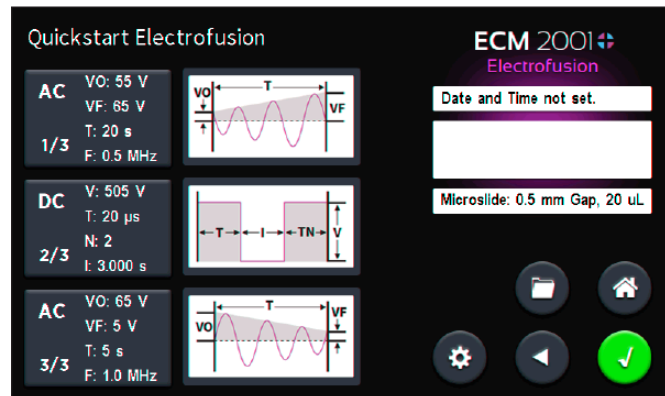
Tap the **Green Check** icon to accept all protocol values.

Software Setup

Electrofusion Quickstart (continued)

Creating Quickstart Method

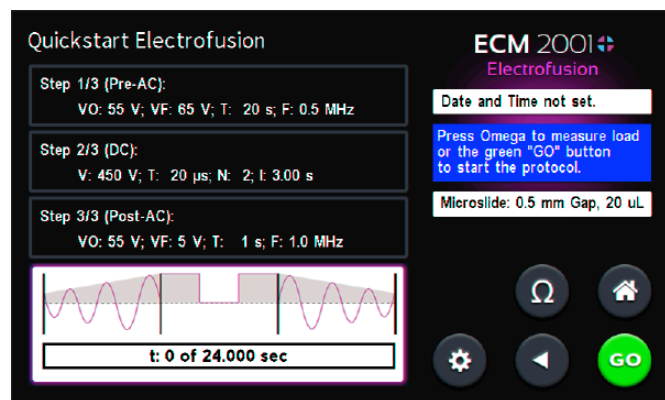
After programing all three steps you will see the following screen:



Each of the values previously programmed for each of the steps can be seen to the left of the graphical representation of each step.

- Tap **Folder** icon to save method for later use.
- Tap **Back** icon to return to the 'Main Electrofusion Menu'.
- Tap **Home Screen** icon to return to the 'Main Electrofusion Menu'.
- Tap **Gear** icon to access unit settings.

Tap **Green Check Icon** to proceed to Electrofusion run screen.



The settings for each of the steps of the method are again summarized in the boxes in the upper left of the screen.

A progress bar is found at the bottom left of the screen with a graphical representation of the method above it.

The time indicated in the progress bar represents the duration of the entire method.

- Tap **Back** icon to return to the previous screen where you can adjust method parameters.
- Tap **Home Screen** icon to return to the 'Main Electrofusion Menu'.
- Tap **Gear** icon to access unit settings.

Tap the **Omega** icon to start a low voltage (approx 17 V) DC pulse (pre-pulse). This is used to check that the conductivity of the sample is not too high for a successful run.

There will be a red failure message screen if the conductivity of the sample is too high.

- Tap **Red Failure Message** screen to acknowledge.
- Use new sample with that has less conductivity.
- Tap **Omega** icon to check the suitability of the sample
- Repeat until successful pre-pulse has been executed

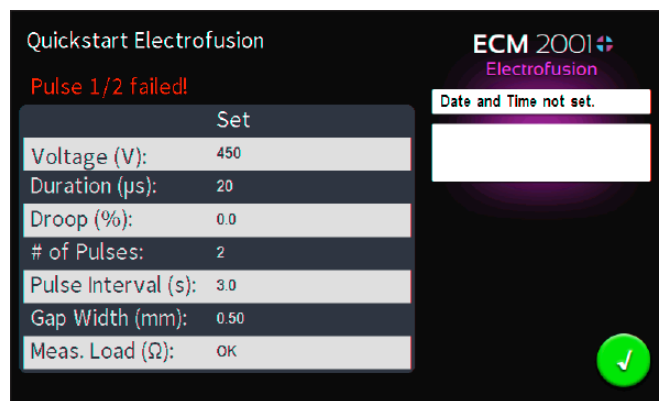
Tap **Green 'Go'** icon to start method.

NOTE: You may tap red 'Stop Icon' to abort the method at any time.

If method failed, a red message will be shown.

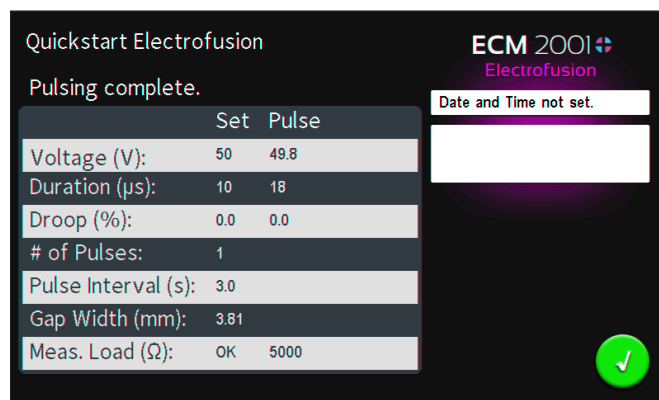
Tap **Red Failure Message** to acknowledge.

Review electrofusion summary screen to see at what step the method failed and to compare the set parameters with the delivered ones to help determine the cause of the failed method.



Tap **Green Check** icon to return to the Electrofusion run screen.

After a successful run, set and measured (delivered) values can be reviewed.

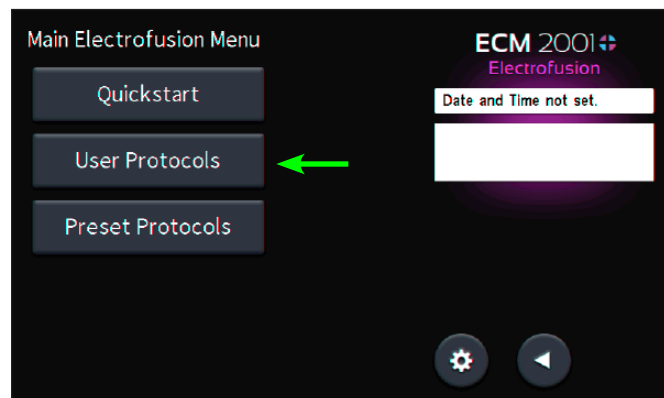


Software Setup

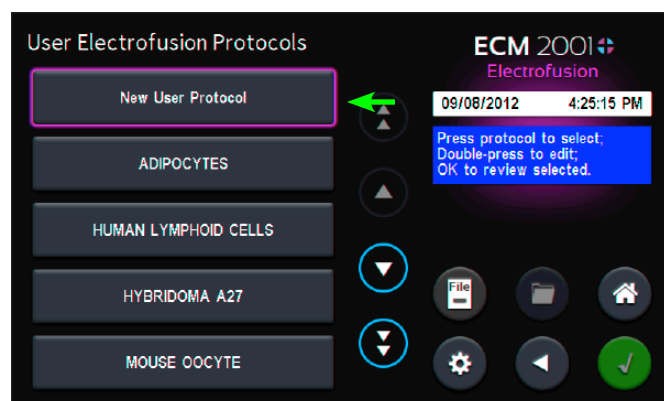
Electrofusion User Protocols

Running User Protocol

Tap **User Protocols**.

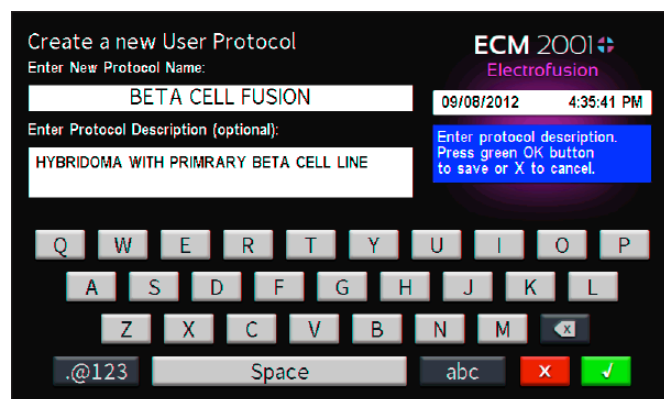


Select **New User Protocol** to create new custom protocol.



Use the keyboard to enter a name for your protocol.

You can add further details in the description box under your protocol name.



Tap the green **Check** icon to accept.

You will then select the accessory to use from the list.



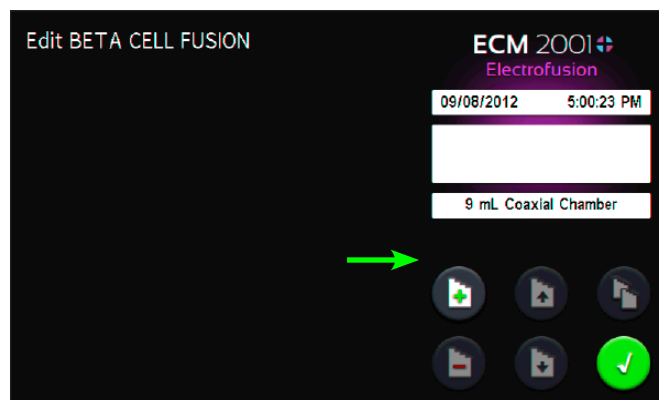
Select accessory from the list. Use up and down arrows to scroll through the list of accessories.

Note: Note: Accessories are listed by item number and description of accessory. Maximum voltage is displayed in blue notification box.

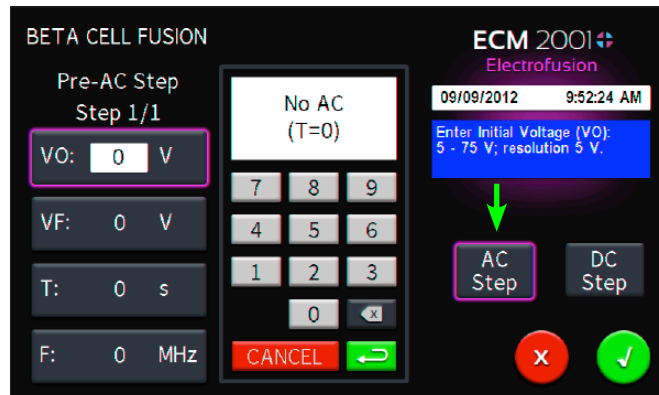
Note: Select 'Other/Unspecified' if using a custom accessory not included on the list. **WARNING: Instrument maximum voltage is permitted with Other/Unspecified; it is the user's responsibility to enter values within the acceptable safe voltage and duration range for their custom accessory to prevent error messages and/or damage to the instrument and accessory. You will be prompted to enter a gap width for 'Other/Unspecified' accessories.**

Tap the green **Check** icon to accept your accessory selection.

Tap the '+' icon to add the first step to your protocol.



You can select **AC Step** or **DC Step**. In this example we have selected to create an AC step.

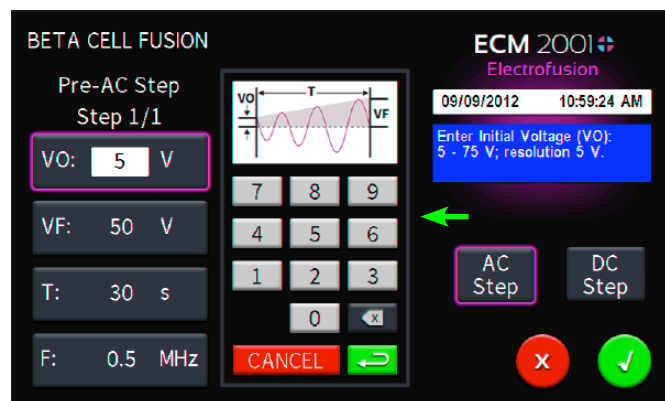


Software Setup

Electrofusion User Protocols (continued)

Creating User Protocols

Use the keypad to enter step parameters. The blue information box will provide guidance for allowable values for each of the parameters.



Note: User protocols can contain from 1 to 20 individual steps.

Note: Each user protocol is limited to one DC step.

Tap the green **Check** icon to accept the parameters for this step in the protocol.

Tap the following icons to add, delete, move or copy steps as needed for protocol:



Add a new step after the selected step.



Delete the selected step.



Move the selected step up a step.

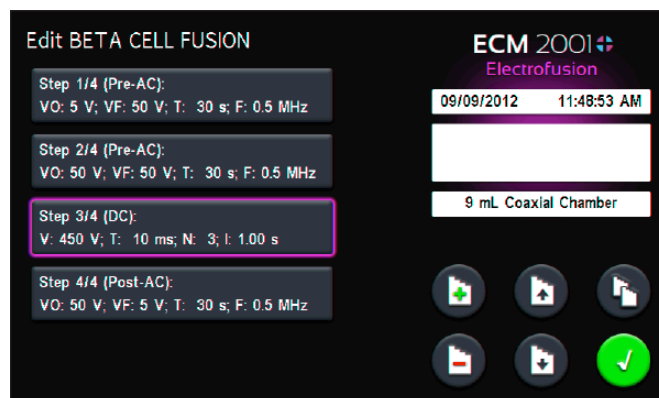


Move the selected step down a step.



Make a copy of the selected step.

Double tap any step to change the parameters in that step.



Tap the green **Check** icon when user protocol is complete.

Software Setup

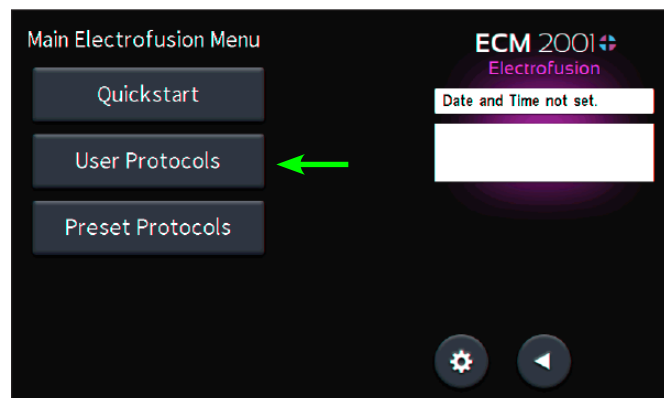
Electrofusion User Protocols (continued)

Modifying Existing User Protocols

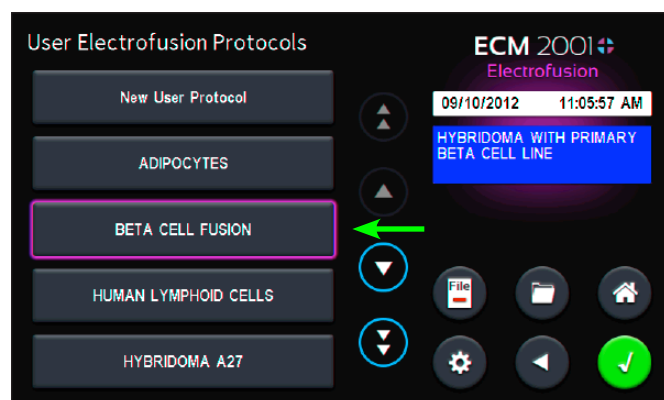
User Protocols can be modified, copied (and used to create a new protocol), or deleted from the system.

Editing Existing User Protocol

Select **User Protocols** from the main electrofusion menu.



Double tap an existing user protocol to open it for editing.



Use these icons to add, delete or reorder steps in the protocol.



Add a new step after the selected step.



Delete the selected step.



Move the selected step up a step.

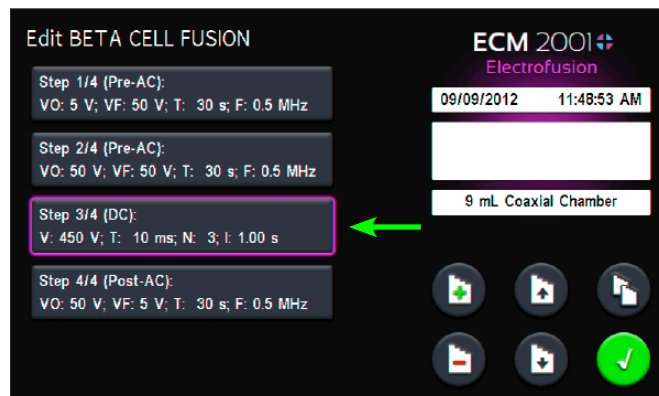


Move the selected step down a step.

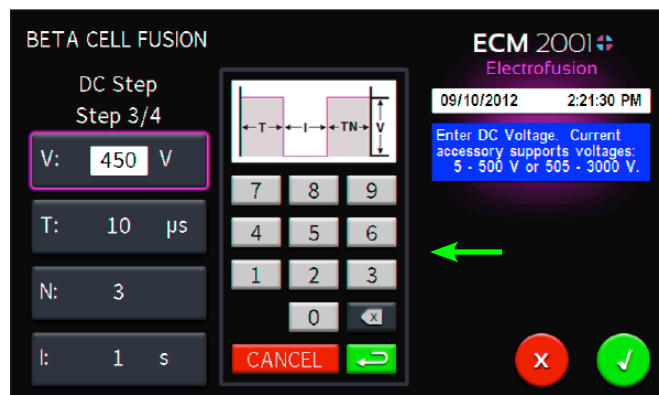


Make a copy of the selected step.

To change the parameters of any step in the protocol, tap the step to be changed.

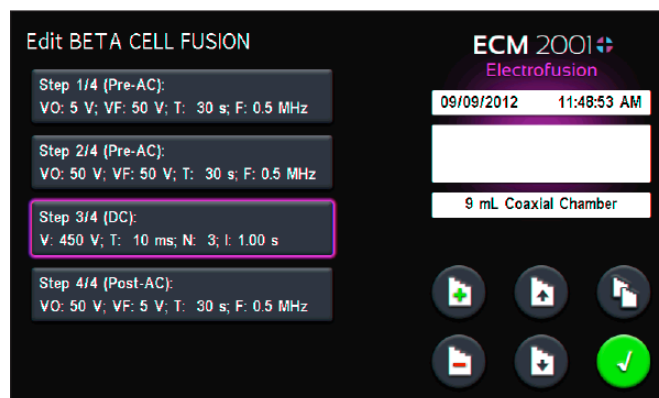


Select the parameter(s) that you would like to change and use the keypad to change values.



Tap the green **Check** icon to accept changes to the step.

Tap the green **Check** icon again to save your changes to the protocol.



Note: The edited 'User Protocol' will replace the original version. To keep the original version, follow the steps in the next section 'Creating a New Protocol from an Existing Protocol'.

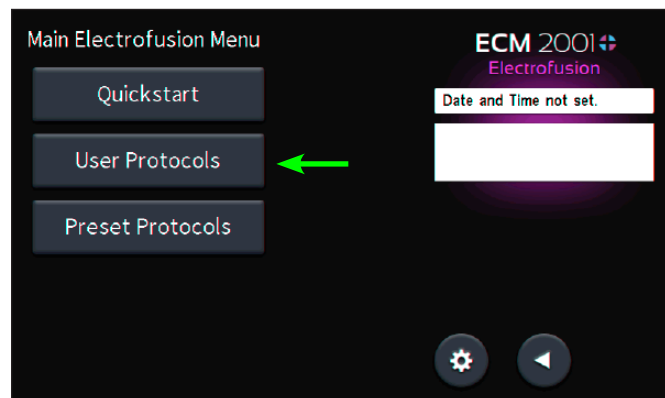
Software Setup

Electrofusion User Protocols (continued)

Modifying Existing User Protocols (continued)

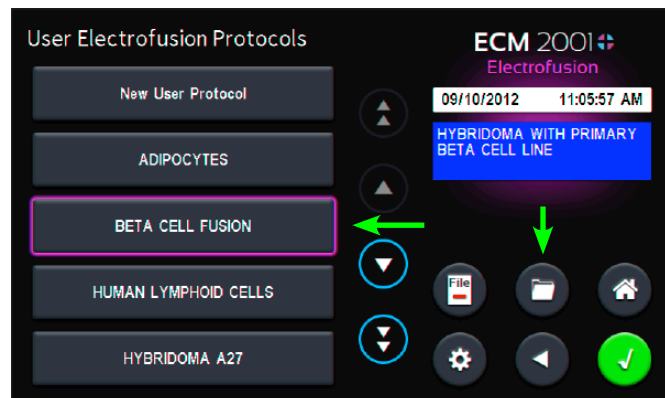
Creating a New Protocol from an Existing Protocol

Select **User Protocols** from the main **Electrofusion** menu.

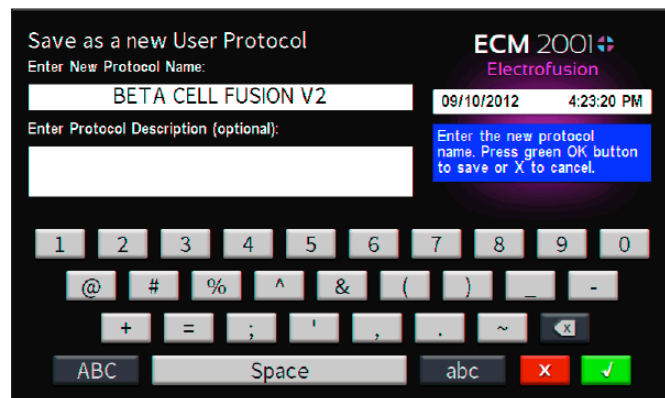


Select **User Protocols** to edit into a new one by tapping it once.

Tap the **Folder** icon to create a copy of the protocol.

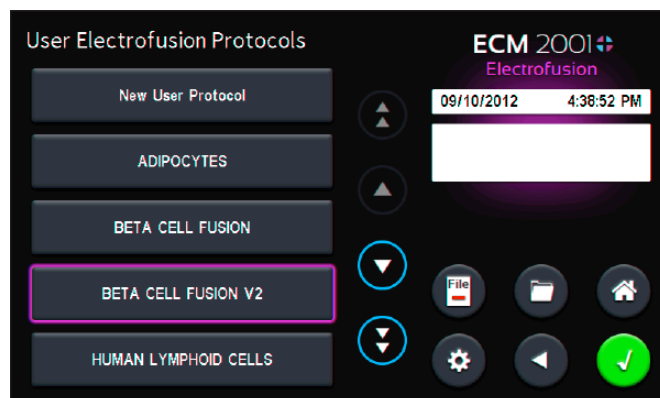


Enter a new protocol name. A protocol description can also be added here (optional).



Tap the green **Check** icon to accept new protocol name.

Double tap the newly-created **User Protocol** to open it for editing.



Use these icons to add, delete or reorder steps in the protocol.



Add a new step after the selected step.



Delete the selected step.



Move the selected step up a step.



Move the selected step down a step.



Make a copy of the selected step.

Tap the green **Check** icon to save your changes.

To change the parameters of any step in the protocol, tap the step to be changed.

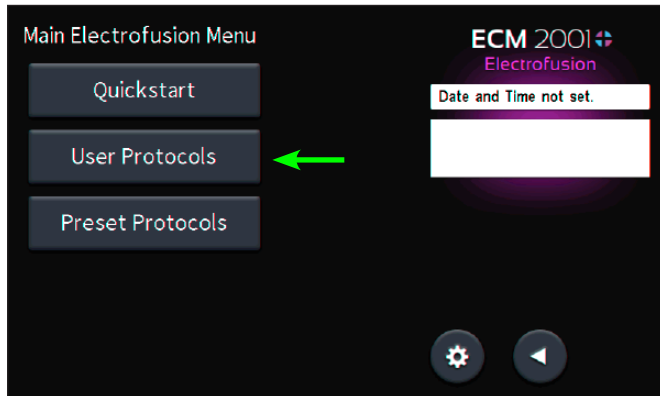
Software Setup

Electrofusion User Protocols (continued)

Modifying Existing User Protocols (continued)

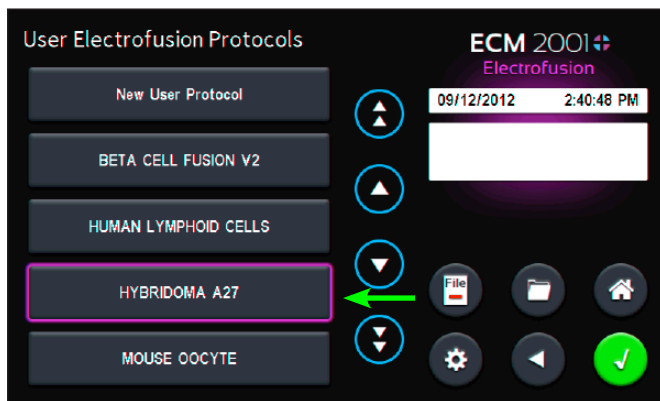
Deleting an Existing User Protocol

Tap **User Protocols**.



Use the up and down arrow buttons to scroll through the **User Protocols**.

Tap on user protocol to be deleted to select it.



Tap on **File** ' ' button to delete selected protocol.



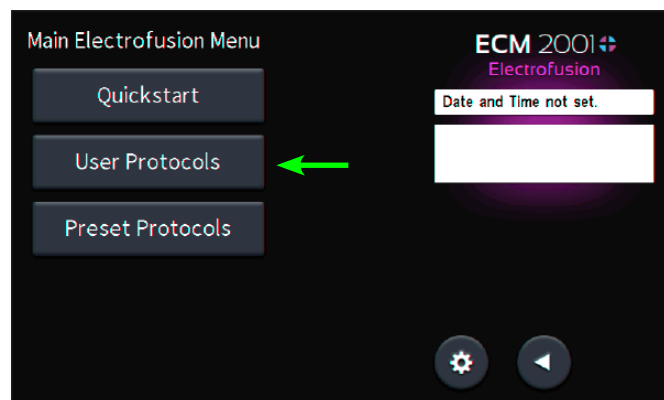
Software Setup

Electrofusion User Protocols (continued)

Modifying Existing User Protocols (continued)

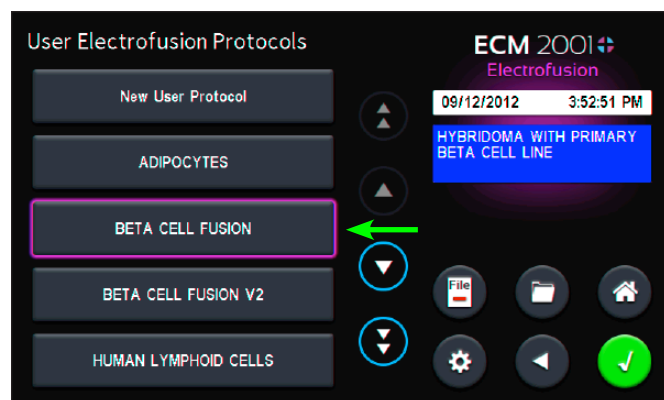
Running User Protocols

Select **User Protocols** from the main electrofusion menu.

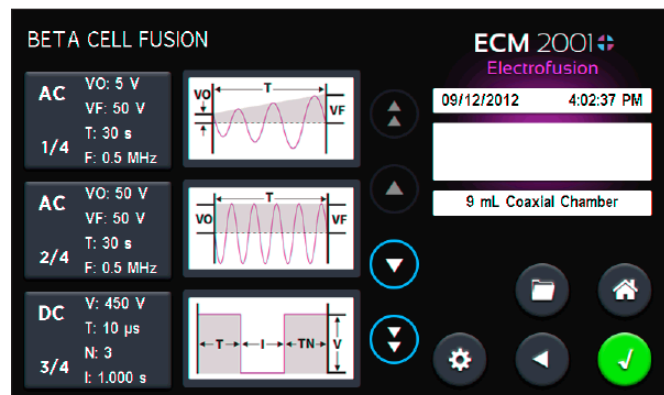


Select **User Protocols** to run by using the up and down scrolling arrows as necessary, then tap the one to be modified to select it.

Tap the green **Check** icon to edit the protocol.

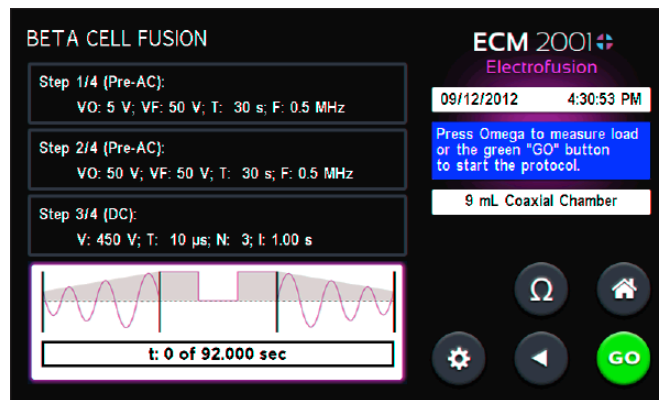


Each of the values previously programmed for each of the steps can be seen to the left of the graphical representation of each step.



Note: If protocol has more than 3 steps, use scrolling arrows to cycle through all of the steps to review all of the steps and their parameters.

Tap green **Check** icon to proceed to **Electrofusion** run screen.



The settings for each of the steps of the method are again summarized in the boxes in the upper left of the screen.

A progress bar is found at the bottom left of the screen with a graphical representation of the method above it.

The time indicated in the progress bar represents the duration of the entire method.

- Tap **Back** icon to return to the previous screen where you can adjust method parameters.
- Tap **Home Screen** icon to return to the 'Main Electrofusion Menu'.
- Tap **Gear** icon to access unit settings.

Tap the **Omega** icon to start a low voltage (approx 17 V) DC pulse (pre-pulse). This is used to check that the conductivity of the sample is not too high for a successful run.

There will be a red failure message screen if the conductivity of the sample is too high.

- Tap **Red Failure Message** screen to acknowledge.
- Use new sample that has less conductivity.
- Tap **Omega** icon to check the suitability of the sample
- Repeat until successful pre-pulse has been executed

Tap Green **'Go'** icon to start method.

Note: You may tap Red **'Stop'** icon to abort the method at any time.

Software Setup

Electrofusion User Protocols (continued)

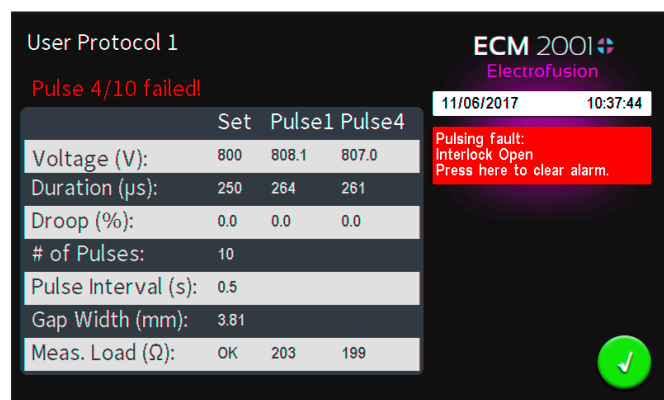
Modifying Existing User Protocols (continued)

Running User Protocol (continued)

If method failed, a red message will be shown.

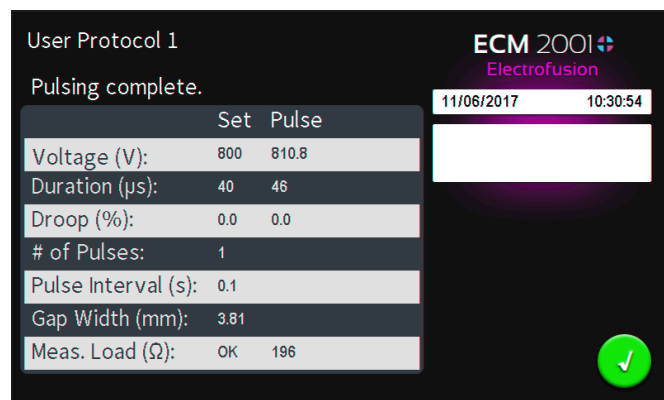
Tap **Red Failure Message** to acknowledge.

Review **Electrofusion** summary screen to see at what step the method failed and to compare the set parameters with the delivered ones to help determine the cause of the failed method.



Tap **Green Check** icon to return to the **Electrofusion** run screen.

After a successful run, set and measured (delivered) values can be reviewed.



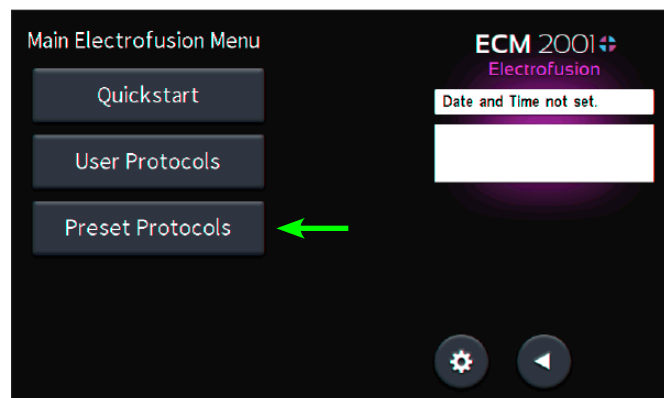
Tap **Green Check** icon to return to the **Electrofusion** run screen.

Software Setup

Electrofusion Preset Protocols

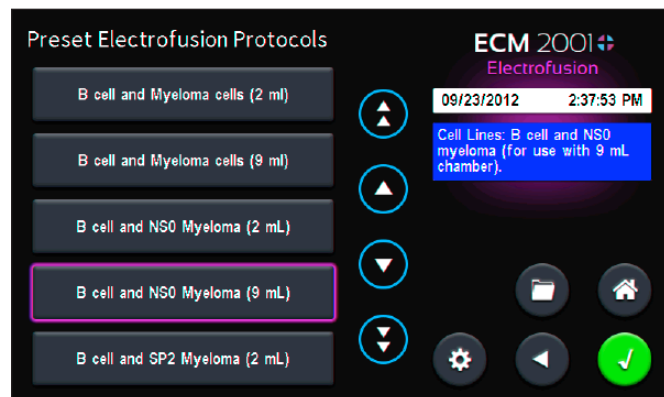
The ECM 2001+ system comes with a number of preset protocols. These may be used directly with no changes or copied and used to create a new **User Protocol** based on the selected **Preset Protocol**.

Tap **Electrofusion** from the home screen and then select **Preset Protocols**.



Use the up and down arrows to scroll through the list of **Preset Protocols**.

Tap the desired protocol once to select it.

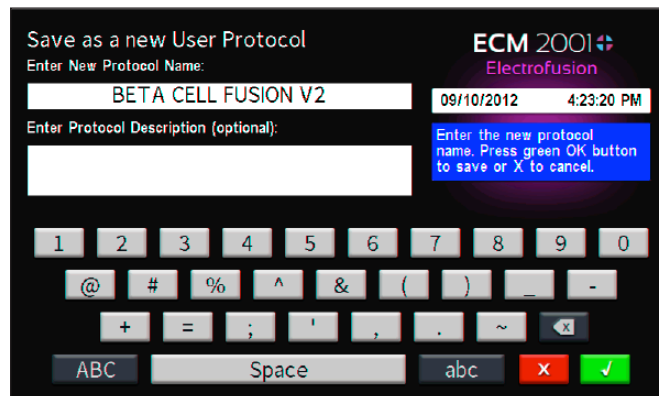


Note: Additional details for the selected **Preset Protocol** will be displayed in the blue box on the right side of the screen.

Customize a Preset Protocol

Tap the **Folder** icon to create a copy of the protocol.

Enter a new protocol name. A protocol description can also be added here (optional).



Tap the **Green Check** icon to accept new protocol name.

Note: The renamed protocol can be found under **User Protocols** where it can be edited.

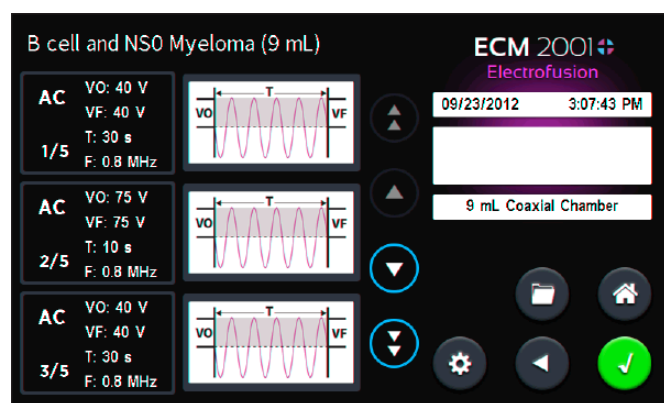
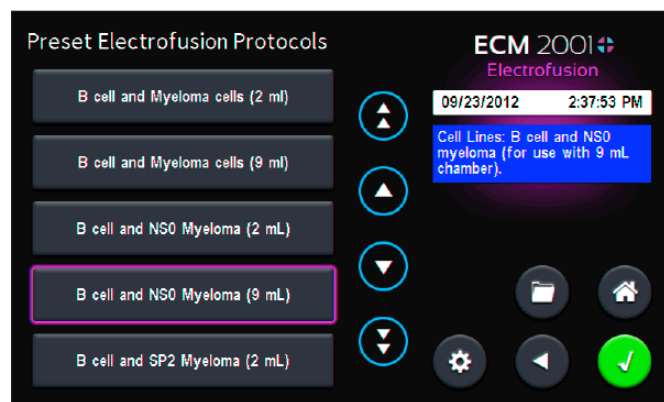
See 'User Protocol' section for details on how to customize the newly named 'Preset Protocol'.

Software Setup

Electrofusion Preset Protocols (continued)

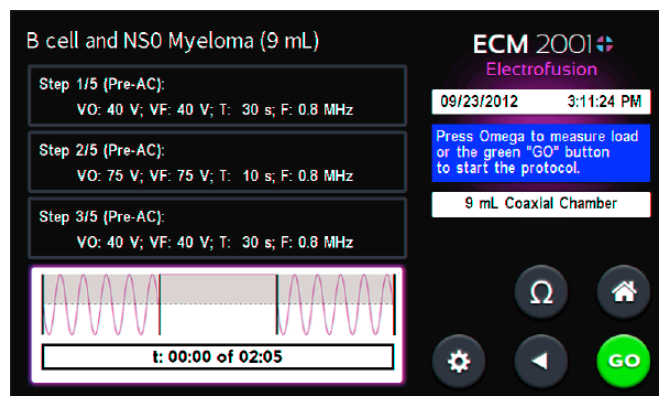
Running Preset Protocol

After selecting **Preset Protocol**, tap the **Green Check** icon.



Note: If protocol has more than 3 steps, use scrolling arrows to cycle through all of the steps to review all of the steps and their parameters.

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



The settings for each of the steps of the method are again summarized in the boxes in the upper left of the screen. A progress bar is found at the bottom left of the screen with a graphical representation of the method above it.

The time indicated in the progress bar represents the duration of the entire method.

- Tap **Back** icon to return to the previous screen where you can adjust method parameters.
- Tap **Home Screen** icon to return to the 'Main Electrofusion Menu'.
- Tap **Gear** icon to access unit settings.

Tap the '**Omega**' icon to start a low voltage (approx 17 V) DC pulse (pre-pulse). This is used to check that the conductivity of the sample is not too high for a successful run.

There will be a red failure message screen if the conductivity of the sample is too high.

- Tap **Red Failure Message** screen to acknowledge.
- Use new sample with that has less conductivity.
- Tap **Omega** icon to check the suitability of the sample
- Repeat until successful pre-pulse has been executed

Tap **Green 'Go'** icon to start method.

Note: You may tap Red '**Stop**' Icon to abort the method at any time.

Software Setup

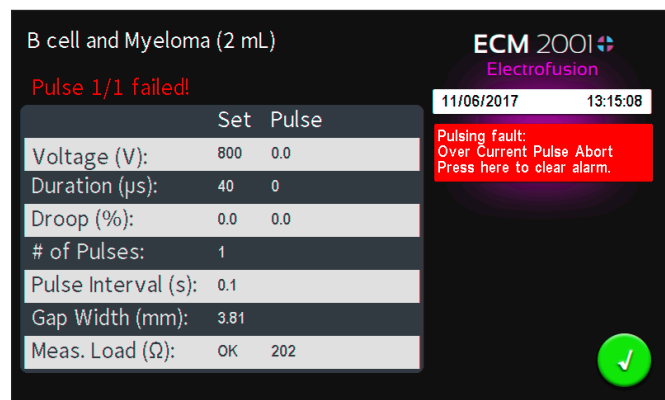
Electrofusion Preset Protocols (continued)

Running Preset Protocol (continued)

If method failed, a red message will be shown.

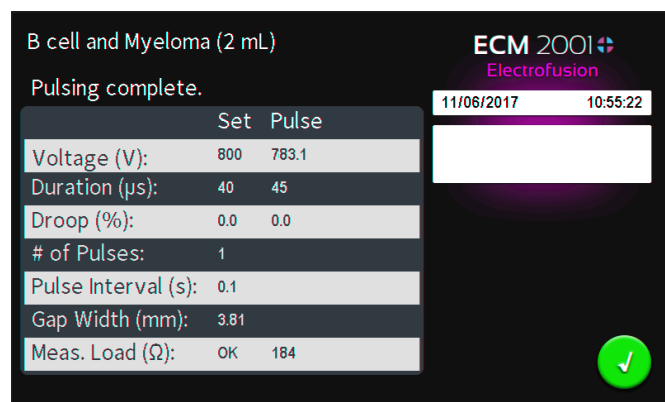
Tap **Red Failure Message** screen to acknowledge.

Review Electrofusion summary screen to see at what step the method failed and to compare the set parameters with the delivered ones to help determine the cause of the failed method.



Tap **Green Check** icon to return to the **Electrofusion** run screen.

After a successful run, set and measured (delivered) values can be reviewed.



Tap **Green Check** icon to return to the **Electrofusion** run screen.

If a footswitch (Item 45-2030) is connected to the Footswitch port at the front of the instrument, then the footswitch may be used for hands-free operation of the instrument.

- From the Protocol Run Screen, a single tap of the Footswitch will initiate the protocol run (similarly to the sequence of pressing Omega and Green Go icons)
- Each additional Footswitch tap will sequentially advance to the next step of the protocol.

Note: If optimal alignment time for desired cell type is unknown, this feature allows the user to set a longer duration than is needed for initial alignment step, then manually advance to the next stage of the protocol when alignment is visualized on a microscope using microslides or 2 ml optimization chamber.

- If the protocol is on the last step, an additional footswitch tap will stop the protocol.

Software Setup

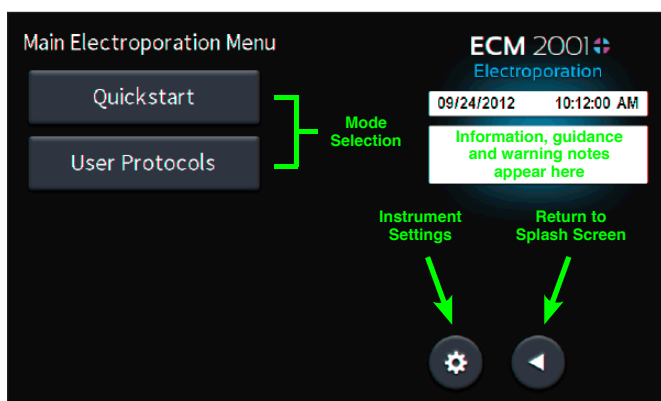
Electroporation Screen Overview

After initializing, the splash screen will appear.

Tap the **Electroporation icon**.



Electrofusion Main Menu



Once selecting 'Electroporation' from the home screen, all screens will have the heading 'Electroporation' in the upper right hand corner to indicate that you are in the Electroporation function of the instrument. This will be changed to 'Electrofusion' when the Electrofusion function has been selected from the home screen.

Mode Selection

User selects the electroporation mode they would like by selecting one of the two icons:

Quickstart	Standard 1-step protocol with guided input depending on the electroporation accessory that has been selected. This protocol allows some flexibility and is suitable for most applications.
User Protocols	Custom protocols that are programmed by the user. Choose this option to conveniently save electroporation protocols for different accessories and/ or conditions. Previously saved custom user protocols can be found here.

Notification Box

This box, located just under the Date and Time banner, provides information on the status of a run, context-sensitive guidance for changing parameters that affect the run and warnings for when conditions and settings are outside the acceptable range for safe use. The following color scheme indicates the type of message and the actions (if any) required.

Information / Status Message

Provides information relevant to the current application or guidance for next steps if appropriate. During protocol programming, ranges of acceptable values will be displayed. There is no user input required for these messages.

Warning Message

This type of message indicates that a condition has arisen that requires User acknowledgment, but does not prevent operation of the system. Warning messages include a user instruction to verify that the protocol parameters are within the specifications for the selected accessory. Failure to take correct action in response to this message could lead to improper operation of the system or damage the accessory. A Warning Message is also used to ask for User confirmation for certain actions, such as deleting a User Protocol. The User must acknowledge Warning Messages by tapping on the Notification Box to clear the message before further action is allowed.

Failure Message

This type of message indicates that there was a failure to complete an operation, and steps must be taken to correct this issue in order to complete the operation. The cause may be a user input error (such as attempting to set a parameter to an invalid value), failure of a protocol, or a system error (such as failure of a hardware component). The Notification Box will contain text and/or a fault code (refer to "Error Messages and Troubleshooting" to help the user diagnose the issue and recover if possible). The User must acknowledge Failure Messages by tapping on the Notification Box to clear the message before further action is allowed.

Instrument Settings

The gear icon allows you to navigate to the **Instrument Settings** from this screen. This sets generator settings for both **Electrofusion** and **Electroporation** functions of the unit.

Return to Splash Screen

This icon takes you back to the home screen where you can select between **Electrofusion** and **Electroporation** functions of the unit.

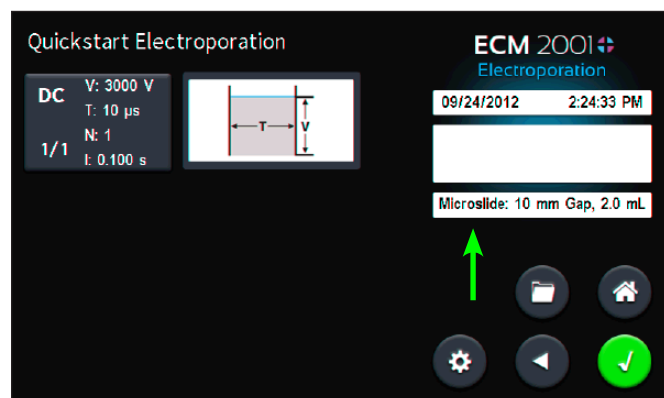
Software Setup

Electroporation Quickstart

Setting DC Parameters

Tap the **Quickstart** icon.

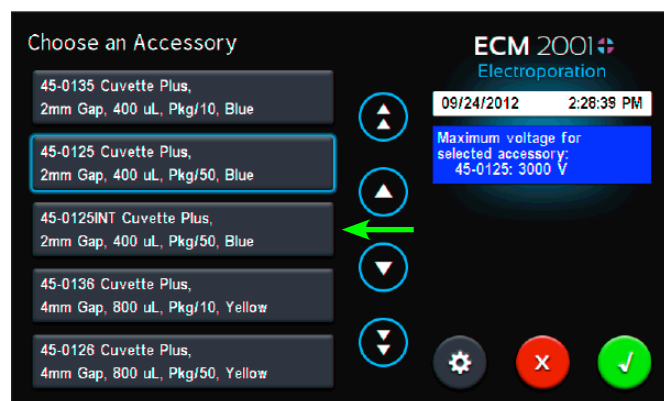
Confirm that the correct accessory is indicated in this window. If not, click on the window containing the accessory description to select a different one.



NOTE: It is important that the correct accessory is selected first. Selecting the accessory after programming protocol settings can result in errors that will prevent you from a successful run.

Use the up and down arrows to scroll through the list of compatible accessories.

Tap on the icon containing the name of the accessory you plan to use.

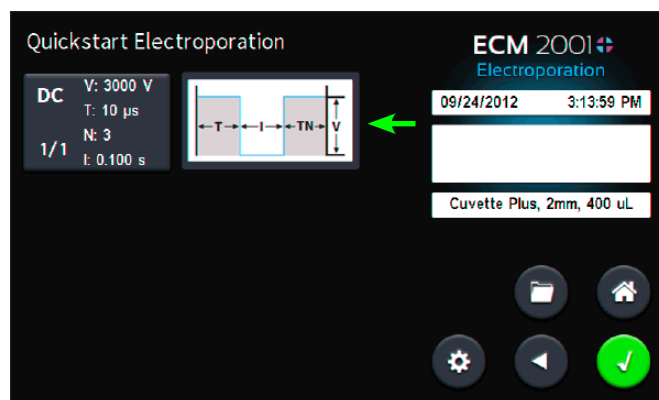


Tap the Green **Check** icon to accept accessory selection.

Tap the top icon (either on the graphic or on the box to the left of it) to edit electroporation parameters.

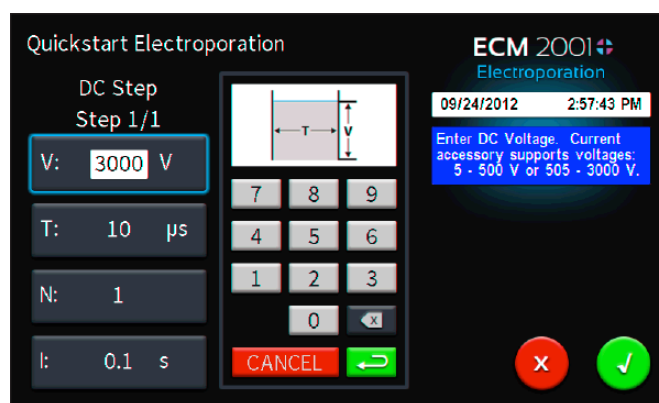
Tap the green **Check Icon** to accept changes.

NOTE: The blue message box will provide you with appropriate ranges and resolutions for the accessory that you selected for this step.

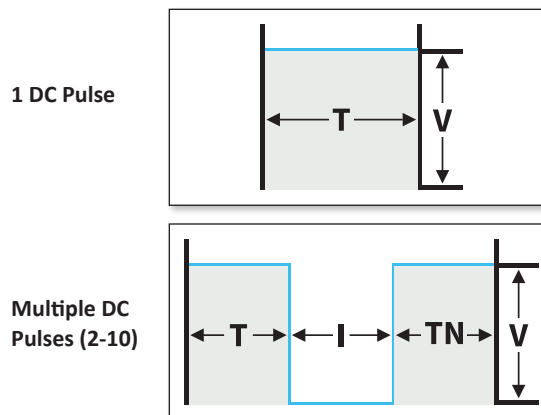


Use the central keypad to enter values for:

- **V:** DC Pulse voltage
- **T:** DC Pulse duration
- **N:** Number of pulses
- **I:** Pulse interval (time between multiple pulses)



The graphical representation of the DC parameters set in this step may change due to your selections. These are not to scale:

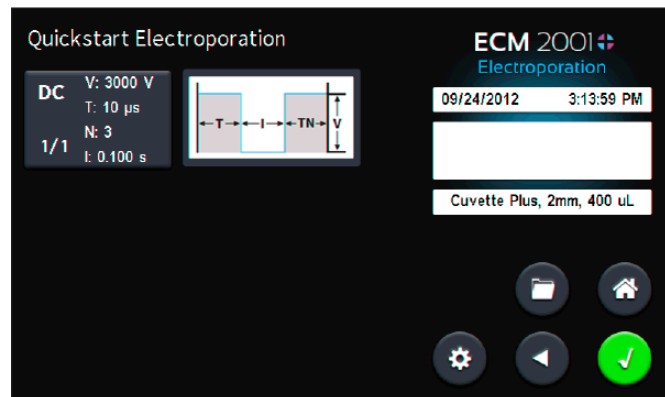


Software Setup

Electroporation Quickstart (continued)

Running Quickstart Method

After programming you will see the following screen:



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

Each of the values previously programmed can be seen to the left of the graphical representation.

- Tap **Folder** icon to save method for later use.
- Tap **Back** icon to return to the 'Main **Electroporation** Menu'.
- Tap **Home Screen** icon to return to the Main Electroporation Menu.
- Tap **Gear** icon to access unit settings.

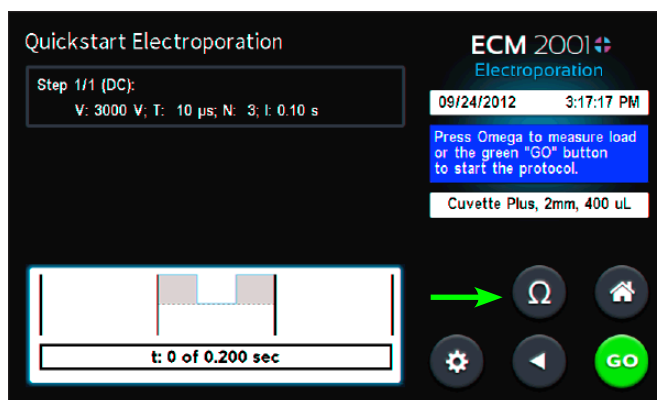
The electroporation parameters of the method are again summarized in the box in the upper left of the screen.

A Progress bar is found at the bottom left of the screen with a graphical representation of the method above it.

The Time indicated in the progress bar represents the duration of the entire method.

- Tap **Back** icon to return to the previous screen where you can adjust method parameters.
- Tap **Home Screen** icon to return to the 'Main Electroporation Menu'.
- Tap **Gear** icon to access unit settings.

Tap the '**Omega**' icon to start a low voltage (approx 17 V) DC pulse (pre-pulse). This is used to check that the conductivity of the sample is not too high for a successful run.



Tap the '**Omega**' icon to start a low voltage (approx 17 V) DC pulse (pre-pulse). This is used to check that the conductivity of the sample is not too high for a successful run.

Software Setup

Electroporation Quickstart (continued)

Running Quickstart Method (continued)

There will be a red failure message screen if the conductivity of the sample is too high.

- Tap **Red Message Screen** to acknowledge.
- Use new sample with less conductivity.
- Tap **Omega Icon** to check the suitability of the sample.
- Repeat until successful pre-pulse has been executed.

Tap **Green 'Go'** icon to start method.

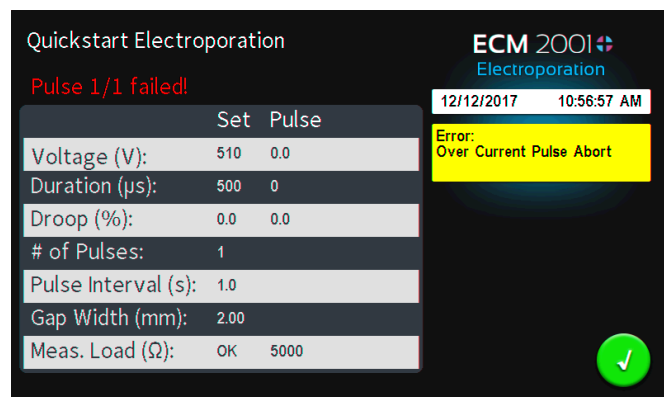
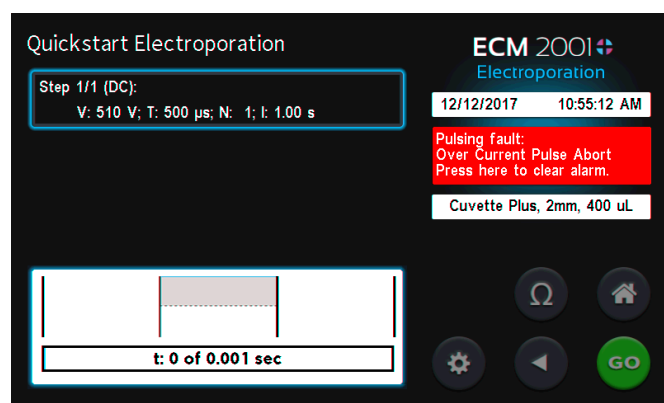
Note: You may tap red 'Stop' Icon to abort the method at any time.

Note: Progress bar will move across to indicate the progress of the method currently being run. The progress bar correlates with the percent of the method that has been completed rather than actual time elapsed.

If method failed, a red message will be shown.

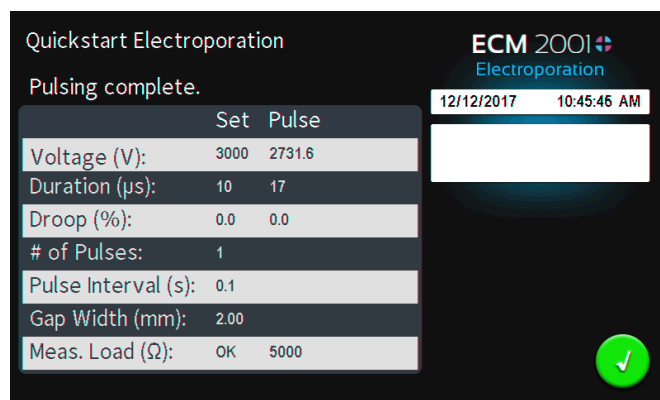
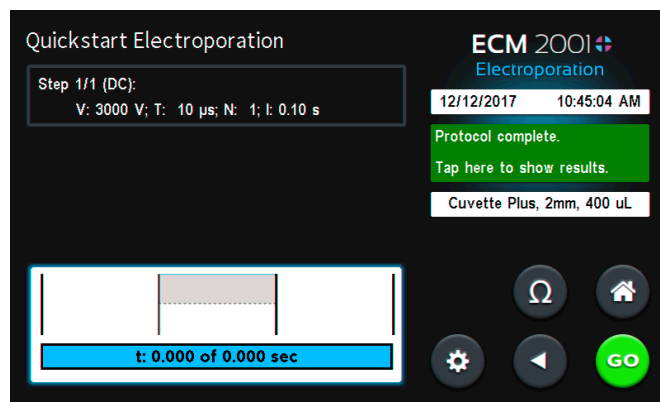
Tap **Red Failure Message** screen to acknowledge.

Review Electroporation summary screen to review data and to compare the set parameters with the delivered ones to help determine the cause of the failed method.



Tap **Green Check** Icon to return to the **Electroporation** run screen.

After a successful run, set and measured (delivered) values can be reviewed.



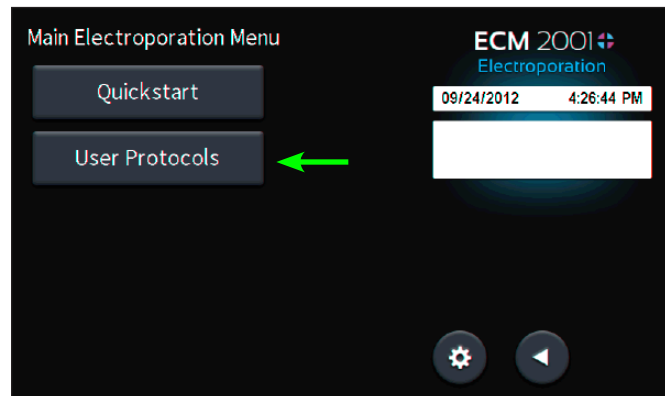
Tap **Green Check** Icon to return to the **Electroporation** run screen.

Software Setup

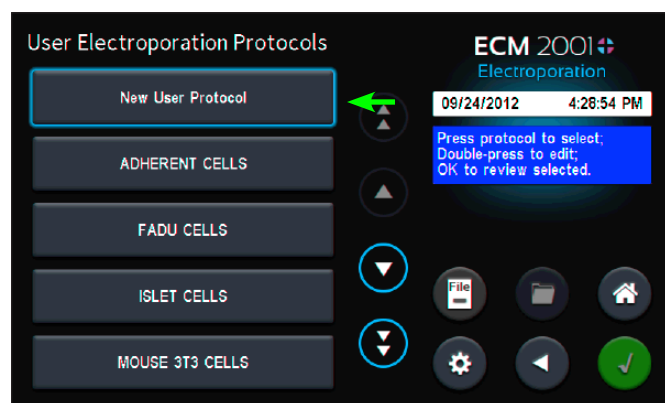
Electroporation User Protocols

Running User Protocols

Tap User Protocols.

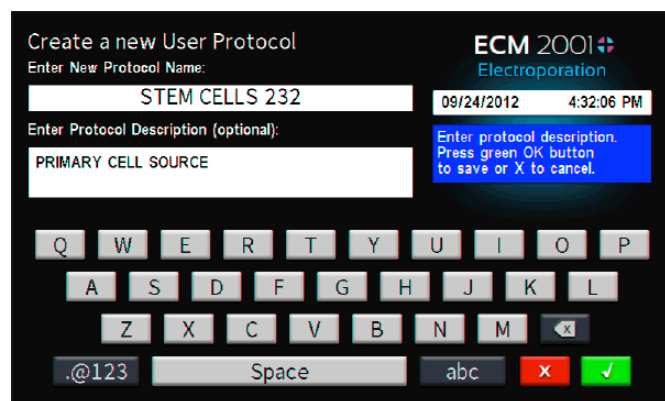


Select **New User Protocol** to create new custom protocol.



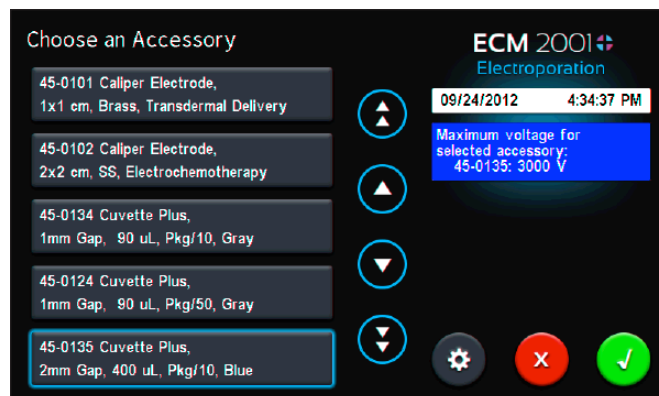
Use the keyboard to enter a name for your protocol.

You can add further details in the description box under your protocol name.



Tap the **Green Check** Icon to accept.

You will then select the accessory to use from the list.



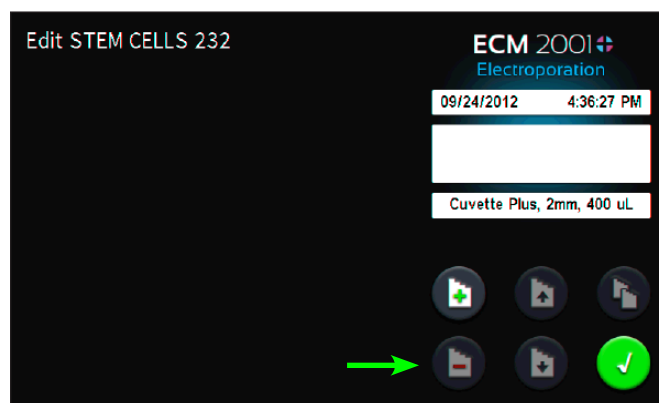
Select accessory from the list. Use up and down arrows to scroll through the list of accessories.

Note: Accessories are listed by item number and description of accessory. Maximum voltage is displayed in blue notification box.

Note: Select 'Other/Unspecified' if using a custom accessory not included on the list. **WARNING: Instrument maximum voltage is permitted with Other/Unspecified; it is the user's responsibility to enter values within the acceptable safe voltage and duration range for their custom accessory to prevent error messages and/or damage to the instrument and accessory. You will be prompted to enter a gap width for 'Other/Unspecified' accessories.**

Tap the **Green Check** Icon to accept your accessory selection.

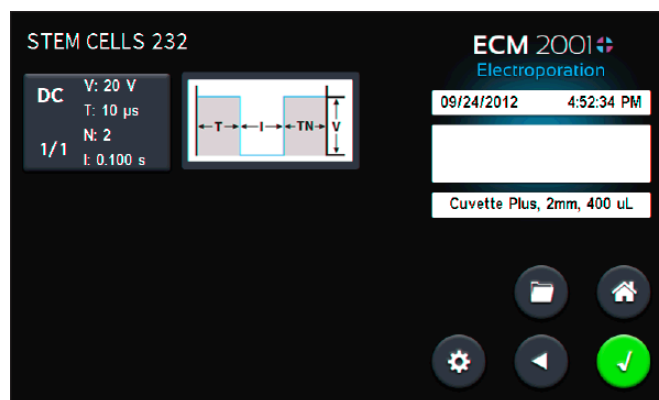
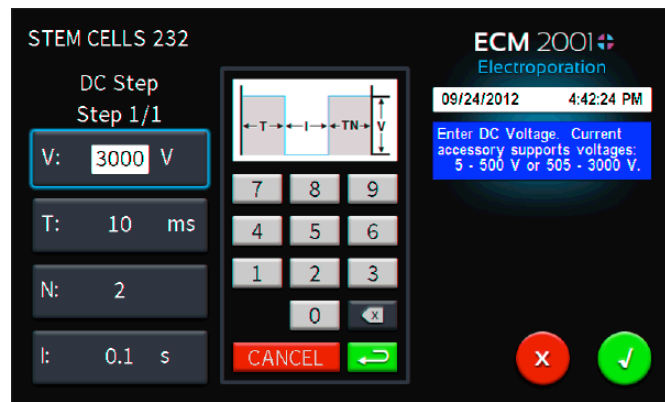
Tap the '+' icon to define your protocol.



Software Setup

Electroporation User Protocols (continued)

Running User Protocols (continued)



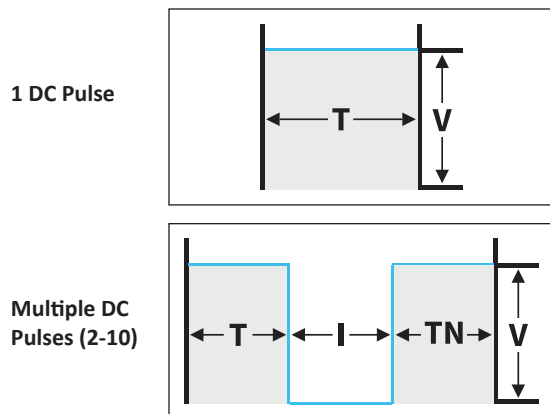
Tap step to edit the parameters in this step.

Tap the **Green Check** Icon when user protocol is complete.

Use the keypad to enter step parameters. The blue information box will provide guidance for allowable values for each of the parameters:

- **V:** DC Pulse voltage
- **T:** DC Pulse duration
- **N:** Number of pulses
- **I:** Pulse interval (time between multiple pulses)

The graphical representation of the DC parameters set in this step may change due to your selections. These are not to scale:



Tap the **Green Check** Icon to accept the parameters for this step in the protocol.

Software Setup

Electroporation User Protocols (continued)

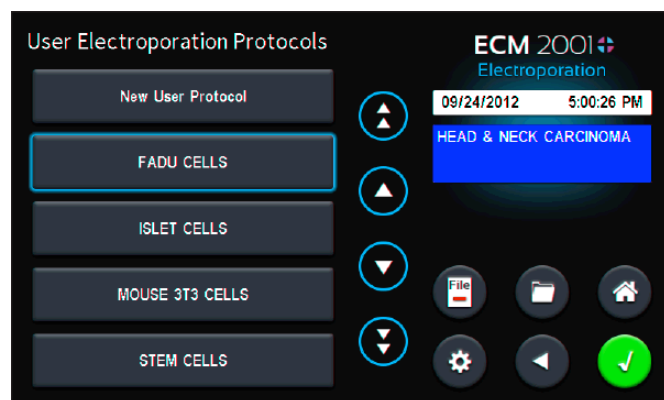
Modifying Existing User Protocols

User protocols can be modified, copied (and used to create a new protocol), or deleted from the system.

Editing Existing User Protocol

Select 'User Protocols' from the Main Electroporation Menu.

Double tap **User Protocols** to open it for editing.



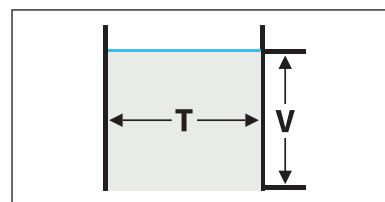
Use the keypad to enter step parameters. The blue information box will provide guidance for allowable values for each of the parameters:

- **V**: DC Pulse voltage
- **T**: DC Pulse duration
- **N**: Number of pulses
- **I**: Pulse interval (time between multiple pulses)

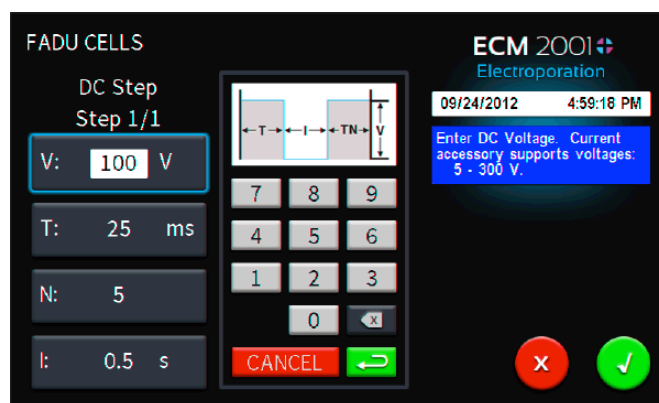
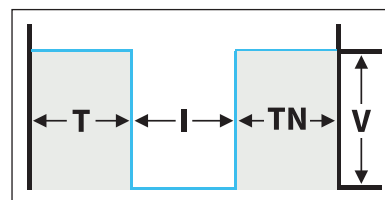
The graphical representation of the DC parameters set in this step may change due to your selections. These are not to scale.

Note: Note: Tapping the DC pulse duration units text (shown here in "μs") toggles the DC pulse duration units between microseconds (μs) and milliseconds (ms).

1 DC Pulse



Multiple DC Pulses (2-10)



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

Note: The edited 'User Protocol' will replace the original version. To keep the original version, follow the steps in the next section 'Creating a New Protocol from an Existing Protocol'.

Software Setup

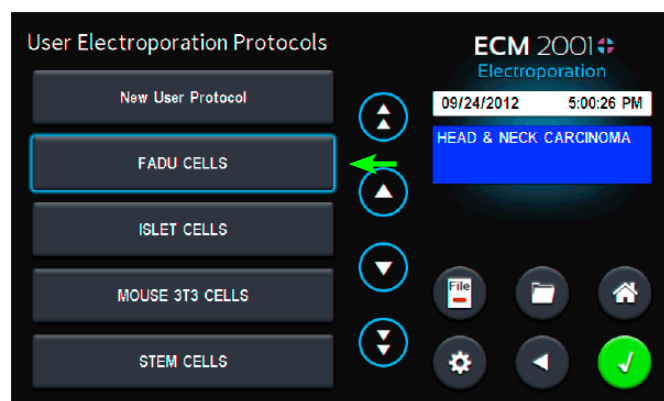
Electroporation User Protocols (continued)

Modifying Existing User Protocols (continued)

Creating a New Protocol from an Existing Protocol

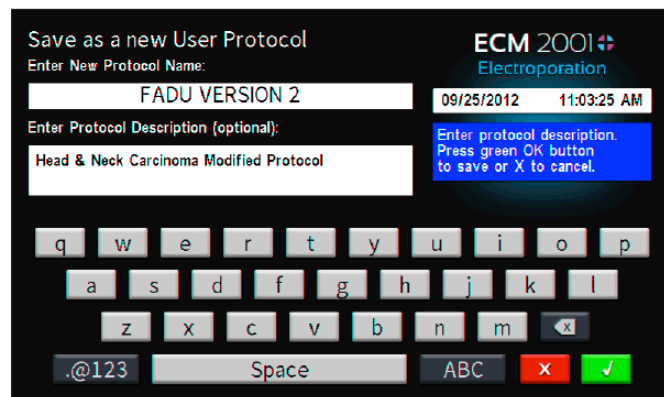
Select 'User Protocols' from the main **Electroporation** menu.

Select user protocol to edit into a new one by tapping it once.



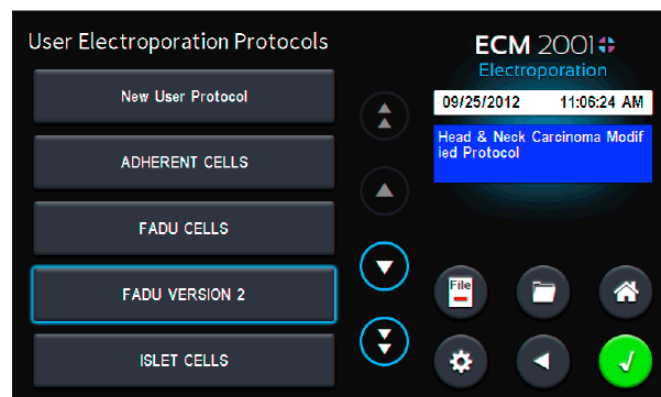
Tap the **Folder** icon to create a copy of the protocol.

Enter a new protocol name. A protocol description can also be added here (optional).

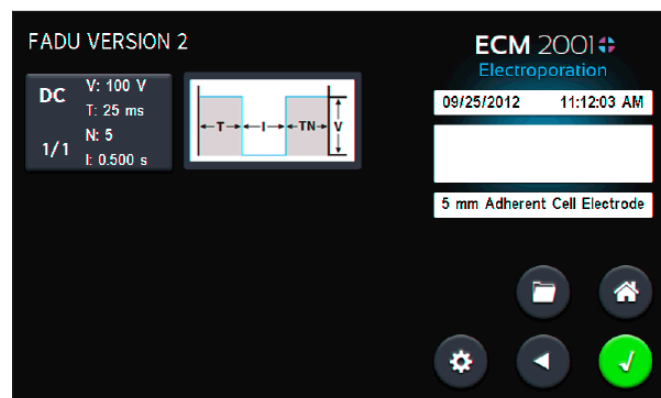


Tap the **Green Check** icon to accept new protocol name.

Select newly named copy of the user protocol and then tap the **Green Check** icon to open it for editing.



Tap the **Accessory** icon to select a different accessory or tap the **Green Check** icon to accept the currently selected accessory.



Note: It is important that the correct accessory is selected first. Selecting the accessory after programming protocol settings can result in errors that will prevent you from a successful run.

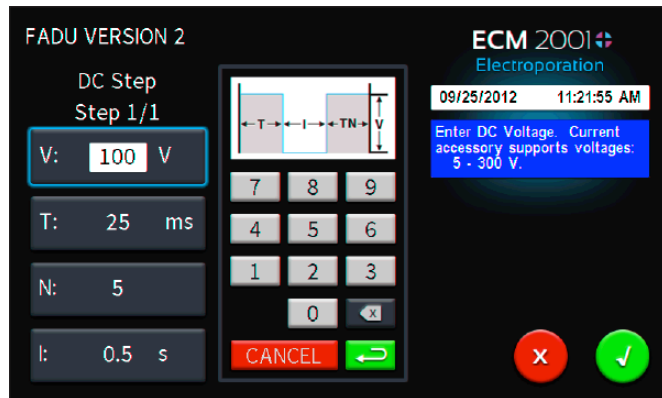
Software Setup

Electroporation User Protocols (continued)

Modifying Existing User Protocols (continued)

Creating a New Protocol from an Existing Protocol (continued)

Tap the graphic or the text box to the left of the graphic of the electroporation protocol to make further edits to the parameters of the run.

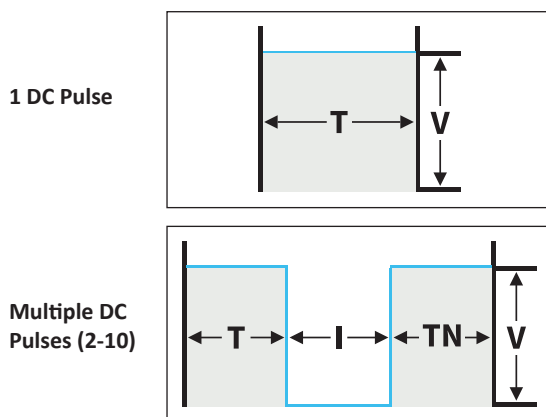


Use the keypad to enter step parameters. The blue information box will provide guidance for allowable values for each of the parameters:

- **V:** DC Pulse voltage
- **T:** DC Pulse duration
- **N:** Number of pulses
- **I:** Pulse interval (time between multiple pulses)

The graphical representation of the DC parameters set in this step may change due to your selections. These are not to scale:

Note: Note: Tapping the DC pulse duration units text (shown here in "ms") toggles the DC pulse duration units between microseconds (μ s) and milliseconds (ms).



Tap the **Green Check** icon to accept the parameters for this step in the protocol.

Software Setup

Electroporation User Protocols (continued)

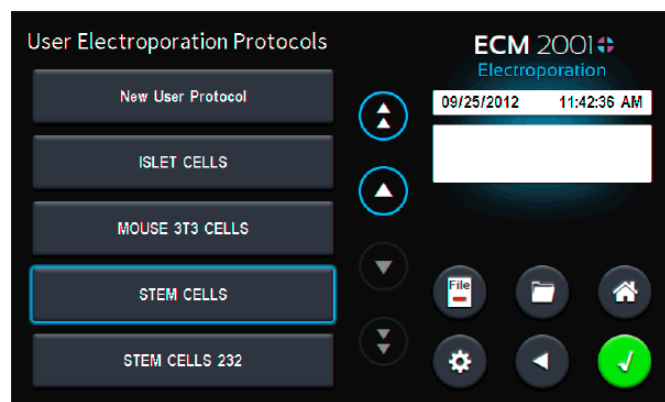
Modifying Existing User Protocols (continued)

Deleting an Existing User Protocol

Tap **User Protocols**.

Use the up and down arrow icons to scroll through the user protocols.

Tap on **User Protocol** to be deleted to select it.

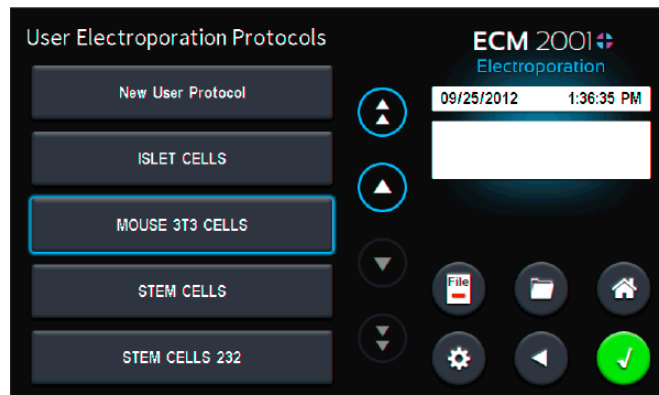


Tap **File** icon to delete selected protocol.

Running User Protocol

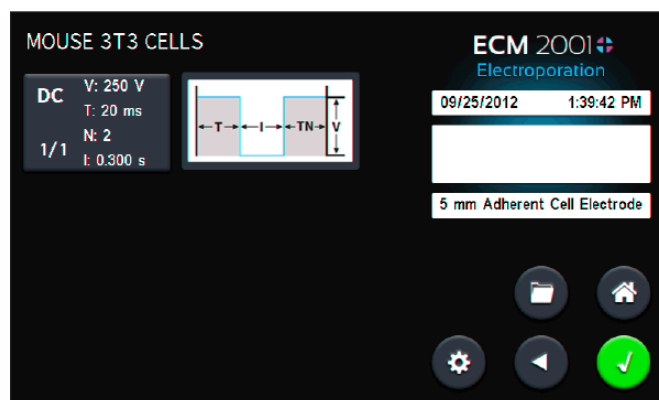
Tap **User Protocols** on the main **Electroporation** menu screen.

Select user protocol to run by using the up and down scrolling arrows as necessary.



Tap the **Green Check** icon to select protocol.

Values for each of the electroporation parameters can be seen to the left of the graphical representation of the protocol.



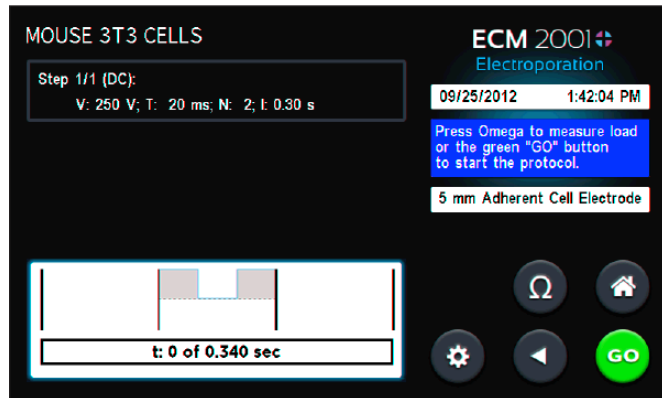
Software Setup

Electroporation User Protocols (continued)

Modifying Existing User Protocols (continued)

Running User Protocol (continued)

Tap green **Check** icon to proceed to **Electroporation** run screen.



The electroporation parameters of the method are again summarized in the box in the upper left of the screen.

A progress bar is found at the bottom left of the screen with a graphical representation of the method above it.

The time indicated in the progress bar represents the duration of the entire method.

- Tap **Back** icon to return to the previous screen where you can adjust method parameters.
- Tap **Home Screen** icon to return to the 'Main Electrofusion Menu'.
- Tap **Gear** icon to access unit settings.

Tap the **Omega** icon to start a low voltage (17V) DC pulse (pre-pulse). This is used to check that the conductivity of the sample is not too high for a successful run.

There will be a red Failure Message screen if the conductivity of the sample is too high.

- Tap **Red Failure Message** screen to acknowledge.
- Use new sample with that has less conductivity.
- Tap **Omega** icon to check the suitability of the sample.
- Repeat until successful pre-pulse has been executed.

Tap **Green 'Go'** icon to start method.

If a footswitch (Item 45-2030) is connected to the Footswitch port at the front of the instrument, then the footswitch may be used for hands-free operation of the instrument.

- From the Protocol Run Screen, a single tap of the Footswitch will initiate the protocol run (similarly to the sequence of pressing Omega and Green Go icons).
- Each additional Footswitch tap will sequentially advance to the next step of the protocol.

Note: If optimal alignment time for desired cell type is unknown, this feature allows the user to set a longer duration than is needed for initial alignment step, then manually advance to the next stage of the protocol when alignment is visualized on a microscope using microslides or 2 ml optimization chamber.

- If the protocol is on the last step, an additional footswitch tap will stop the protocol.

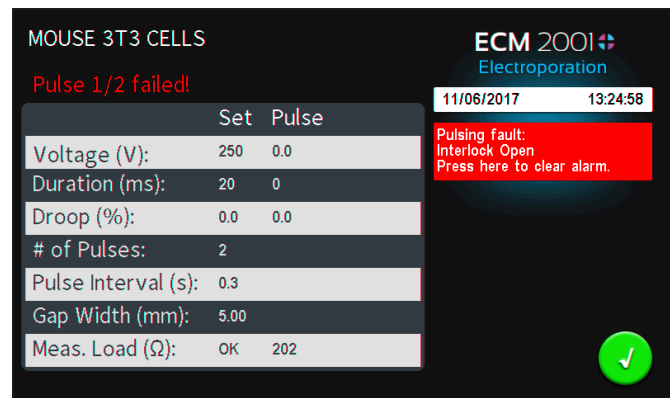
Note: You may tap Red 'Stop' icon to abort the method at any time

Note: Progress bar will move across to indicate which step of the method is currently being run. The progress bar correlates with the percent of the method that has been completed rather than actual time elapsed.

If method failed, a red message will be shown.

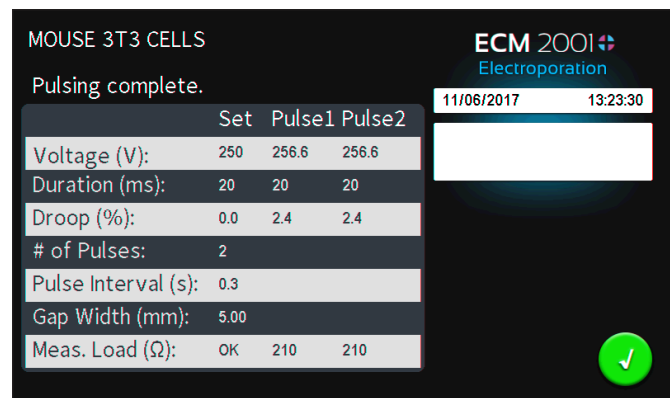
Tap **Red Failure Message** to acknowledge.

Review Electroporation summary screen to review data and to compare the set parameters with the delivered ones to help determine the cause of the failed method.



Tap **Green Check** icon to return to the Electroporation run screen.

After a successful run, set and measured (delivered) values can be reviewed.



Tap **Green Check** icon to return to the Electroporation run screen.

Software Setup

Protocol Manager Software

Installation

Right click on 'Protocol Manager'. Select 'Send To' then 'Desktop' to create a shortcut on your desktop.

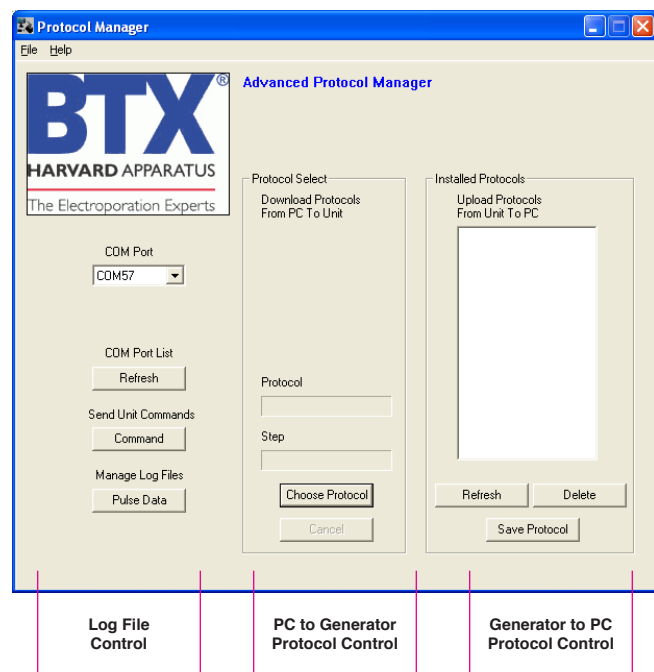
1. Connect the generator to the PC using a USB cable.
2. Turn generator on.
3. Start Protocol Manager.
4. Select the proper COM port.

Note: Compatible with Windows XP, 7, 8, and 10.

Overview

Protocol Manager allows the user to:

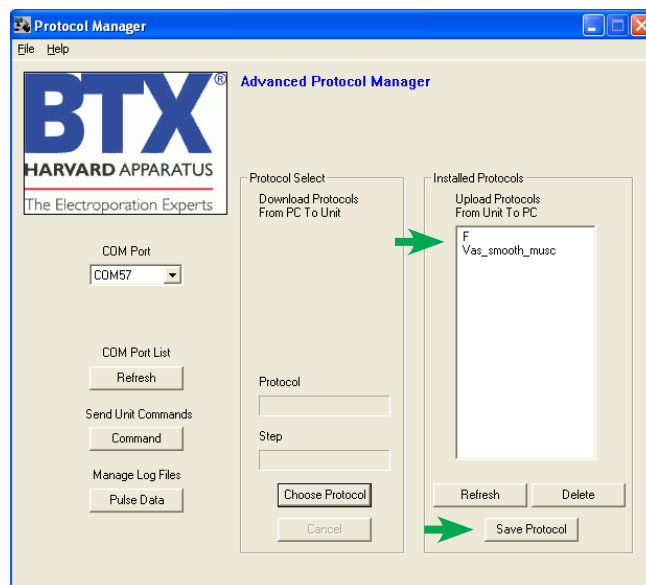
- Upload protocols from the ECM 2001+ generator to a PC.
- Download protocols from PC to the ECM 2001+ generator.
- Upload log files from the ECM 2001+ generator to PC.



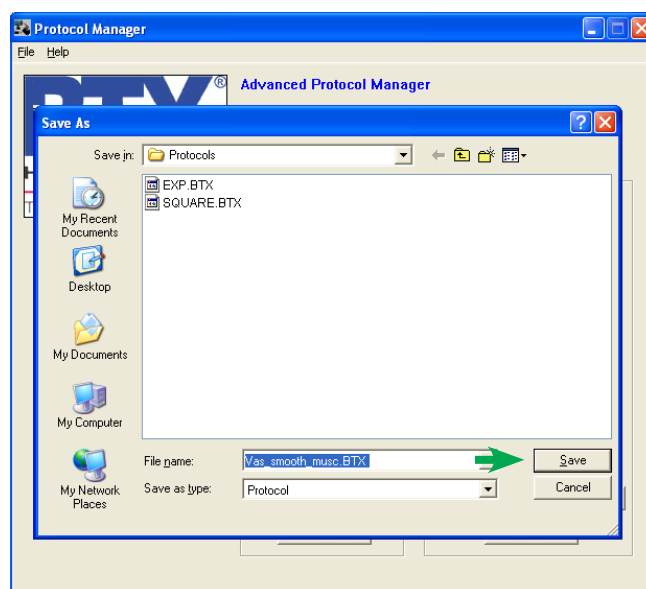
Upload from Generator to PC

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

Note: Hit 'Refresh' to update the list after creating or editing protocols on the ECM 2001+ generator.



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

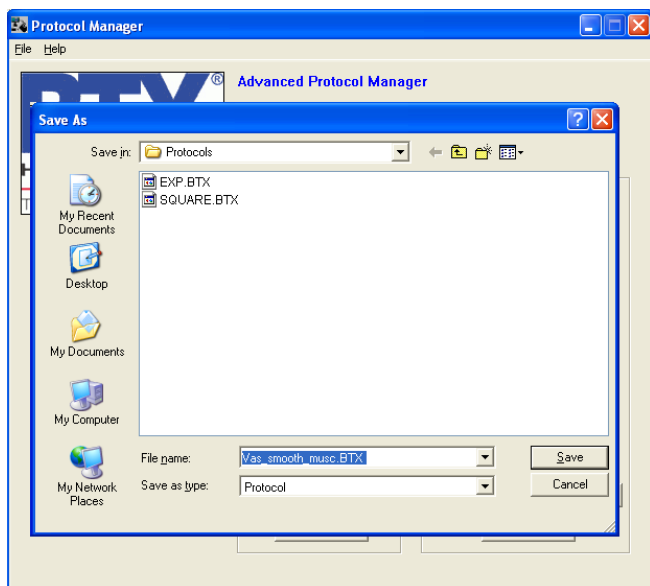


Software Setup

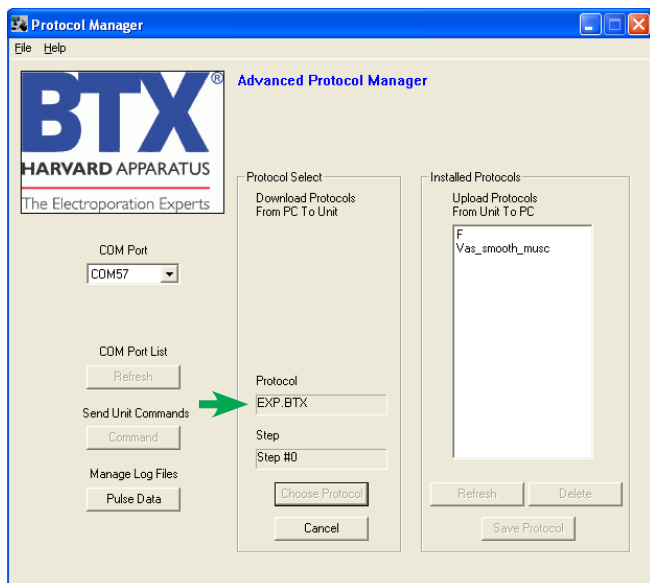
Protocol Manager Software (continued)

Download from PC to Generator

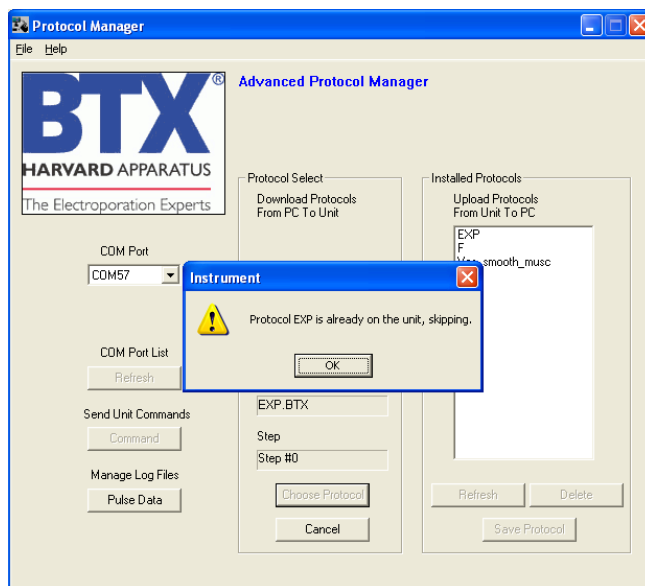
1. Click 'Choose Protocol'.
2. Select protocol(s) to download.
3. Click 'Open'.



4. Protocol name being downloaded is shown.



Note: If a protocol already exists on the ECM 2001+ generator, Protocol Manager will not overwrite the file if you attempt to re-download it. When determining if a protocol already exists, upper vs lower case is ignored. For example, a protocol named "ABC" will be treated as a duplicate of protocol "abc".



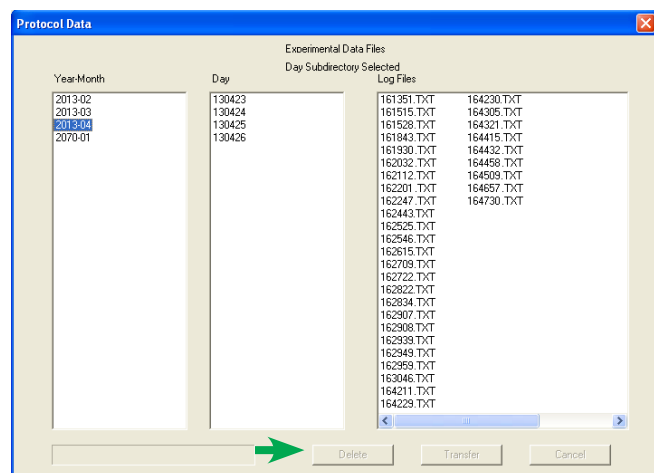
Software Setup

Protocol Manager Software (continued)

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 home screen.
2. Order of Operations:
 1. Connect USB.
 2. Turn generator on.
 3. 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.

	A	B	C	D	E	F	G	H	I	J	K	L
1	Date (MM/DD/YYYY)	5/17/19	Time (HH:MM:SS)	16:00:43	Model	BTX ECM 2001+	Mode	Electroporation				
2	Serial Number	G4510102	Protocol Type	Square Wave	Accessory	Other/Unspecified	Gap (mm)	1.1.0	2			
3	Protocol Name	Quickstart Electroporation	GUI Firmware	2.0.8	DC Firmware	2.0.8	AC Firmware					
4	Auto-PrePulse	Off	Step	Step Type	Step Parameters	T (us)	# of Pulses	Pulse Interval (s)				
5	1	DC				25	100	10	5			
6	10	DC Pulses										
7	11	Voltage (V)				25.1	25.1	25.1	25.1	25.1	25.1	25.1
8	12	Duration (us)				100	100	100	100	100	100	100
9	13	Drop (%)				0	0	0	0	0	0	0
10	14	Load (Ohm)				N/A	N/A	N/A	N/A	N/A	N/A	N/A
11	15	Status Code				0:00	0:00	0:00	0:00	0:00	0:00	0:00
12	16	Protocol passed										
13	17											
14	18											
15	19											
16	20											
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Software Setup

Remote Control Software

Installation

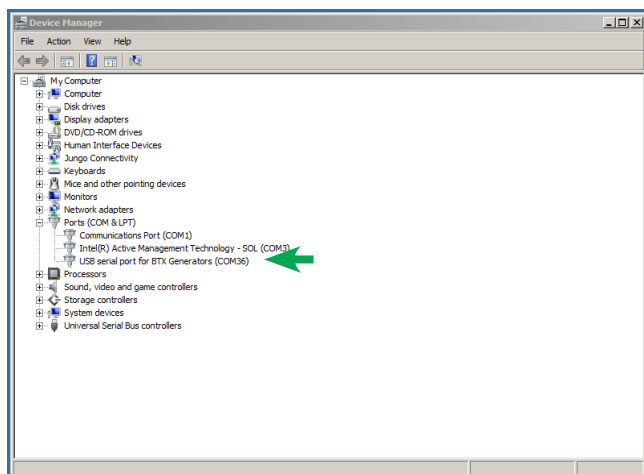
Navigate to the **Remote Screen Interface** folder, and then to the **Remote Screen Interface Support Files Installer** folder. Run the “setup.exe” file. Follow the instructions provided on the installation screens. After the installation is complete, copy the folder **Remote Screen Interface Application** from the CD onto the PC in a location of your choice.

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.

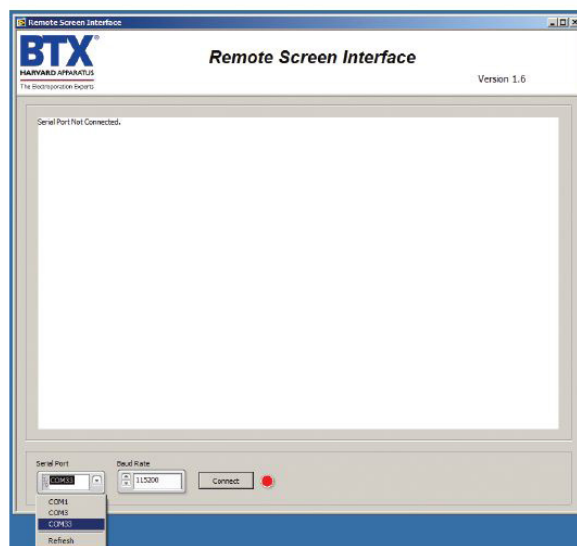
Overview

Remote Screen Interface allows you to control the ECM 2001+ remotely. When you click on the computer screen, you control the generator.

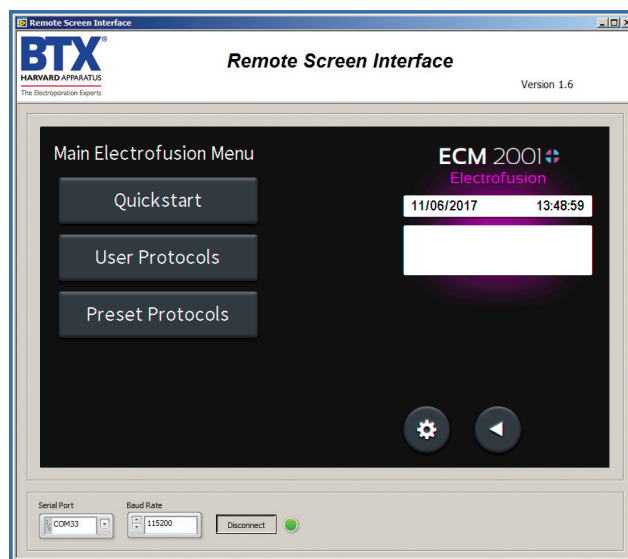
While the ECM 2001+ generator is connected to the computer via USB, open the Control Panel and select **System and Security**, and then **System**. Click **Device Manager** on the left hand menu. Under Ports, search for the “USB serial port for Generators.” This will show the COM Port to which the generator 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 2001 generator remotely.



Specifications

ECM 2001+	
Square Wave Pulse , DC	
Voltage Range	
LV Mode	5 to 500 in 1 V steps
HV Mode	505 to 3000 in 1 V steps
Voltage Accuracy	
LV Mode	5%
HV Mode	5%
Pulse Length Range	
LV Mode	10 to 999 μ s in 1 μ s steps or 1 to 999 ms in 1 ms steps
HV Mode	10 to 600 μ s in 1 μ s steps
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 in 0.01 s steps
Alignment (Pre-Pulse), AC	
Frequency	0.2 to 2 MHz in 0.1 MHz steps
Voltage	5 to 75 V in 5 V steps
Duration	0 to 99 s in 1 s steps
Wave Shape	Sine wave
Post-Fusion, AC	
Frequency	0.2 to 2 MHz in 0.1 MHz steps
Voltage	5 to 75 V in 5 V steps
Duration	0 to 99 s in 1 s steps
Wave Shape	Sine wave
Sample Load Ranges	
AC	
All Voltages	Load must be $\geq 60 \Omega$
DC	
LV Mode	Pulse Length <100 ms, Load must be >8 to 9Ω ; Pulse Length >100 ms, Load must be $>100 \Omega$
HV Mode	Load must be $\geq 40 \Omega$

Other Specifications	
Charging Time	LV = 7 s, HV = 7 s
DC Monitoring	Protocol Name, Steps Executed, Time/Date, Error Conditions, Pulse Voltage, Pulse Current, Pulse Width, Pulse #, Droop %, Sample Voltage, Sample Current and Sample Resistance
Display	7-inch full color display
Controls	Touch Screen
Programmability	Storage over 1000 Protocols
DC Arc Control	Yes for DC
Pre-Pulse Sample Load Check	User-selectable (17 V DC)
Computer (Optional)	PC running Microsoft® Windows XP, 7, 8.1 or 10
Communications	USB
Data File Format	Tab Delimited text
Remote Operation	Yes using Foot Pedal Control
Accessory Connector Type	Coaxial
Power Ratings	70 W idle and 900 W pulsing
Input Voltage Ratings	100 to 240 VAC, 50 / 60 Hz
Dimensions (H x W x D)	13 x 12 x 13 in
Weight	22 lb
Warranty	2 years
Atmospheric Specifications	
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 BTX ECM 2001+ square wave generator requires no special maintenance other than keeping it clean.

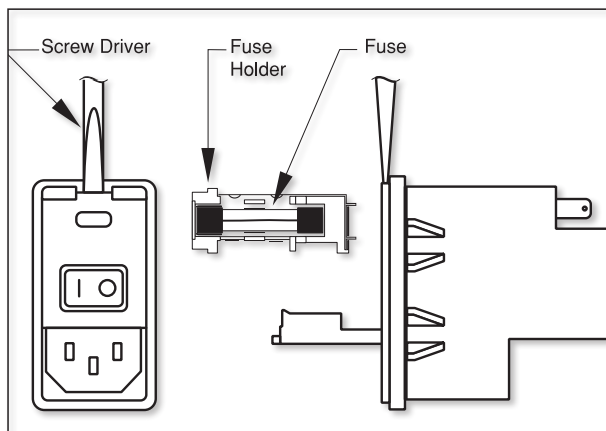
To clean the exterior surfaces, use a lint-free cloth to remove loose dust. Use care to avoid scratching the clear display window. For more efficient cleaning, use a soft cloth dampened (not soaked) with an aqueous solution of 75% isopropyl alcohol, or a mild detergent.

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

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, 16 mm CR1620 by sliding under clip with positive side facing out.

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



Error Messages & Troubleshooting

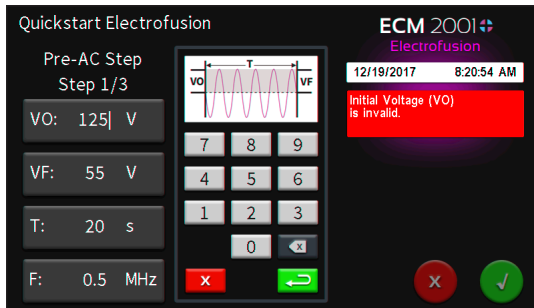
Display Error Message	Error Description	User Troubleshooting
Power Loss Error	Power loss during pulsing.	Power lost while protocol was running.
PFC Over Temp	PFC module over temperature.	Hardware error. Please contact the manufacturer.
Interlock Open	Electrode interlock open.	Connect Interlock Cable
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: 1. Increasing the gap size. 2. Reducing the volume. 3. Use 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: 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.
Resistance Out Of Range	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.
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: 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.
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.
AC Fault: Max AC current exceeded!	AC aborted due over current	AC current was aborted due to current exceeding maximum safe output specifications of the instrument. Note: The current limit that was exceeded will be displayed in milliamps. Ways to resolve this issue: 1. Increase the sample resistance by reducing sample volume or replacing with less conductive buffer 2. Increase gap size. 3. Reduce AC voltage and/or duration.

Error Messages & 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.



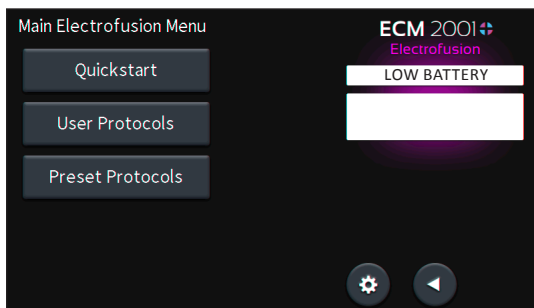
Power Failure Notification

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.



Low Battery Indication

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



Error Messages & 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%.

Cells Don't Align

- Make sure the equipment is set up correctly. All cables should be connected.
- Are cells of good viability (90 % +), free of microbial contamination, and in logarithmic growth phase? If not, change cells.
- Try varying the voltage while observing the cells on the Microslide or 2 mL coaxial chamber on the microscope. Make sure the microscope is focused on cells between wires of microslide or between electrode walls of coaxial chamber.
- Make sure all traces of cell culture media are removed by washing cells 3 times in fusion medium prior to fusion.
- If using Cytofusion Medium C, verify that it is not past its expiration date and within pH specifications.
- With self-prepared fusion medium, check the pH and verify it is between 4 to 7.3. Adjust with 0.1 N NaOH. If too much NaOH is added this produces a basic condition and an undesirable electrical field.
- Optimize cell density. Increase the cell concentration if cells move but do not align. Decrease the cell concentration if the cells are too densely packed to move.
- Work efficiently. For best results minimize the time that cells are sitting in fusion medium and immediately start electrofusion protocol after loading cells into chamber. Do not allow cells to settle and clump at the bottom of fusion chamber.

Cells Don't Fuse

- Make sure the equipment is set up correctly. All cables should be connected. Also verify the instrument is programmed correctly to desired electrofusion settings.
- Check cable connection to fusion chamber and verify continuity e.g. with an ohmmeter.
- Do cells rotate? If yes, optimize alignment amplitude and time.
- Verify pH of fusion medium and minimize time of cells in fusion medium as described in above Alignment section.
- Increase fusion pulse amplitude and pulse length. Cells will lyse at a critical upper setting; stay below this setting.
- Add fusion facilitators: one drop of 1 mg/ml pronase or one drop of 5 mg/ml trypsin to cell suspension about to be fused (30 seconds before fusion.)
- Add or increase duration of post fusion AC confinement force.

Cells Don't Divide

- Reduce instrument settings for alignment amplitude and duration and/or the fusion DC pulse amplitude and pulse length. Verify cell viability with samples pre- and post-fusion with a viability stain such as trypan blue.
- Reduce pronase/trypsin concentration and/or treatment time of cells.
- Post fusion care is critical for cell division. Verify procedure.

Cells Don't Secrete Antibody

- Reduce instrument settings for alignment amplitude and duration and/or the fusion DC pulse amplitude and pulse length. Verify cell viability with samples pre- and post-fusion with a viability stain such as trypan blue.
- Reduce pronase/trypsin concentration and/or treatment time of cells.
- Immunization protocol may have been ineffective, i.e. was animal hyperimmunized? Verify immunization procedure.
- Myeloma cells should be of good viability (90 % +), free of microbial contamination, and in logarithmic growth phase. If not, change cells.
- Verify post fusion care procedure for cells.

Upgrade Information

Upgrading ECM 2001+ Series Software

USB Virtual CommPort Driver Installation

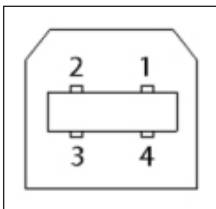
Drivers may be downloaded from the BTX website at:

<https://www.btxonline.com/technical-resources/downloads.html>

When you connect the BTX ECM 2001+ 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.

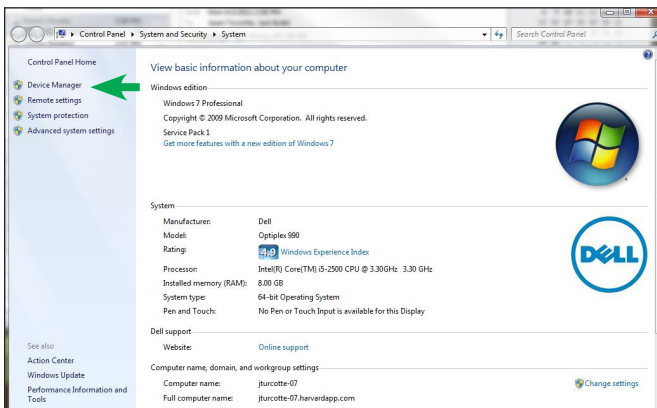
1. Connect the ECM 2001+ generator to the computer via USB. Open the control panel and select System and Security and then System. Click **Device Manager** on the left hand menu.

Mating Face

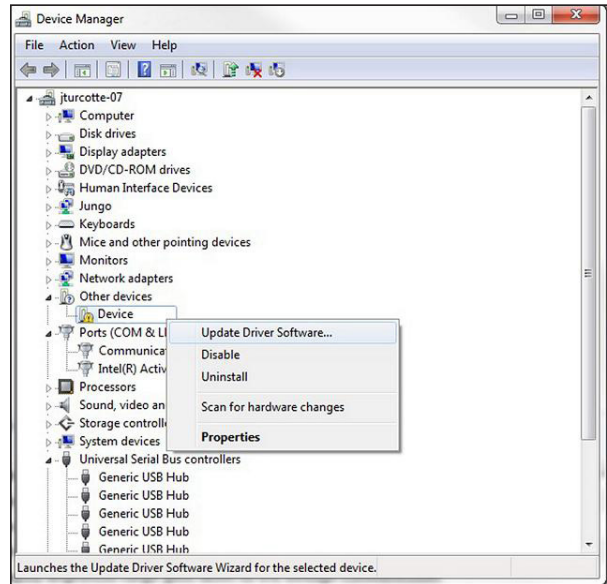


Pin # Signal

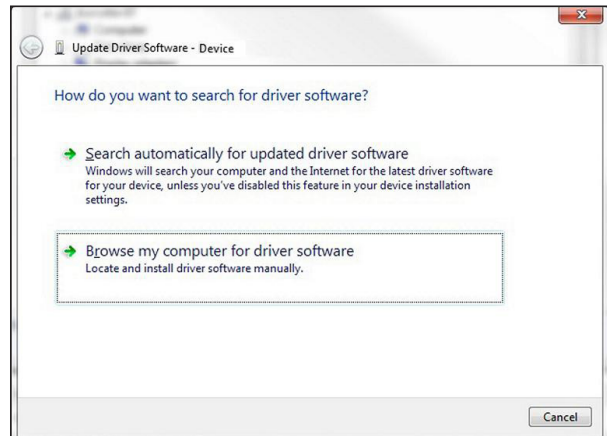
- 1 +5V
- 2 - Data
- 3 + Data
- 4 GND



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**.



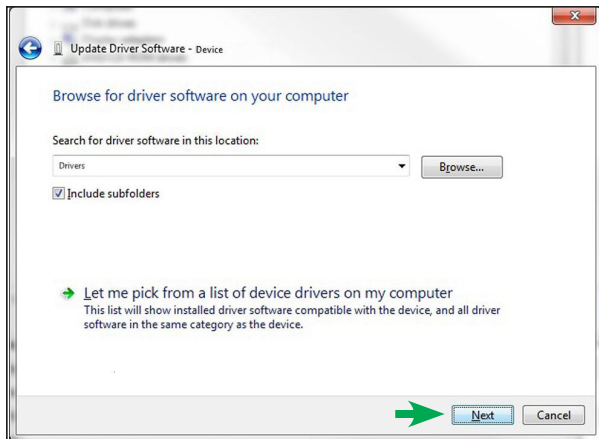
3. Select **Browse my computer for driver software**.



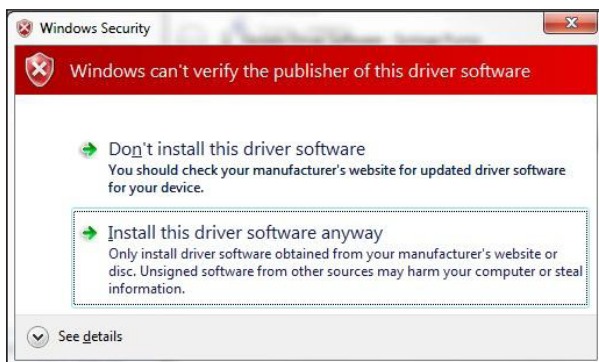
Upgrade Information

USB Virtual Commport Driver Installation (continued)

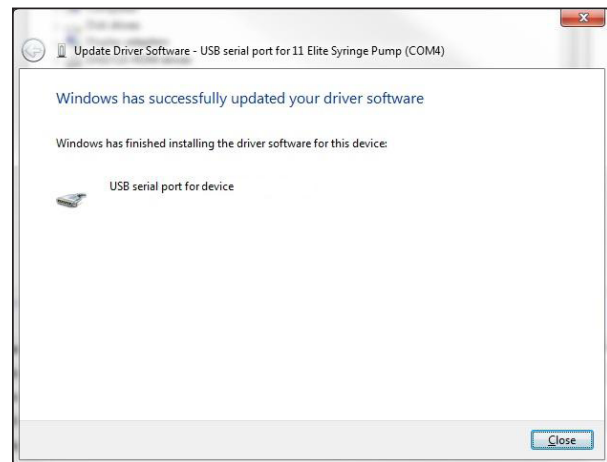
- Click **Browse** and navigate to the generator drivers folder that you have downloaded to your PC from the BTX website. Make sure the **Subfolders** box is checked and then click **Next**.



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



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



Upgrade Information

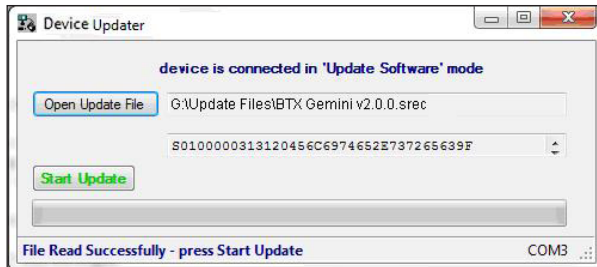
Device Updater Program

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

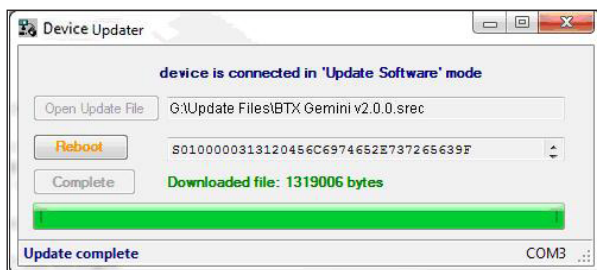
1. Upload the latest software versions to your desktop (format is filename.srec). There is one .srec files that needs to be updated: 'App-BTX-2001+-x.x.x.srec'.
2. Disconnect all I/O devices and then connect the ECM 2001+ generator to the PC using a USB cable.
3. On the Settings screen of the ECM 2001+, press **Update Software** icon then press **Upgrade Software** icon twice to enter **Boot Loader Mode**.

NOTE: To exit Boot Loader mode without upgrading the software, power cycle the ECM 2001+.

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



5. Click Open Update File and browse to the 'App-BTX-2001+-x.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 2001+ generator from the power switch in the back of the unit.

Cell Electrofusion Tutorial

Applications

There are three common uses of electrofusion:

- **Hybridoma production as one step in monoclonal antibody production**
- **Producing therapeutic hybrids for immunotherapy**
- **Nuclear transfer from one cell cytoplasm to another cell cytoplasm**

Hybridoma Production

One step in monoclonal antibody production is producing hybridomas. The following are the basic concepts for making antibody producing hybridomas:

1. Standard myeloma cells are B cell cancer cells that are immortal. They have several important modifications. One is that they have been made susceptible to being killed by specific chemicals that do not kill B cells. Another is that they generally do not produce antibody on their own.
2. In humans and animals there are millions of different types of B cells (lymphocytes) with different antibody specificities. They are not immortal. B cells expand in number in response to external events, stimulation by antigen and T cells.
3. A hybridoma is produced by fusing an immortal B cell line (myeloma) that does not secrete antibody with B cells that have been extracted from blood. The fused cells take on characteristics of both cells (the myeloma's immortality and the B cell's specific antibody).
4. The resulting hybrid is cultured in a special medium that kills unfused myeloma cells while unfused B cells die naturally over time.
5. The hybrids are then placed in a 96-well plate with a dilution where approximately one antibody secreting hybridoma exists per well. After some growth, antibody is detected in the culture supernatant. Cells from positive wells are cloned.

A figure of merit is the number of productive wells with antibodies and the number of cells required to give, on average, a positive well. The number of functional, antibody-secreting, hybridomas is less than the number of fusions.

An example of electrofusion versus PEG was produced by M. Coccia, Ph.D., Platform Development Group at Medarex, Inc., Milpitas, CA. Transgenic human Ab-producing mice were used in experiments comparing efficiencies of electrofusion to PEG fusion. Each experiment used a portion of the same splenocyte preparation from mice immunized with tetanus toxoid (TT) for comparison of the electrofusion and PEG fusion methods. Results shown are the number of TT antigen-specific clones generated by each method normalized to the same number of cells.

These data are representative of additional experiments (not shown) utilizing four different antigens. Taken together, all experiments showed electrofusion generated approximately ten-fold more antigen specific antibody clones relative to PEG fusion.

Experiment Number	Antigen Specific	
	Electrofusion	PEG
1	20	0
2	10	0
3	400	23
4	151	21
Mean	145	11

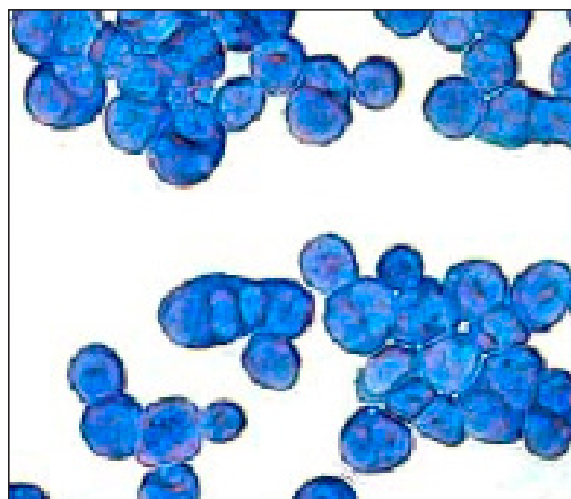


Figure 1: Medarex Fusion Example

Figure 1 shows Cytospin™ prepared and Wrights-Giemsa stained SP2/0 cells 30 minutes following large scale electrofusion. In these electrofusion experiments, more than 75% of cells in the final population were fused with 2 to 3 nuclei per cell.

Another process using human B cells is described in Li, et al., 2006, *PNAS* 103 (10): 3557-3562.

Cell Electrofusion Tutorial

Immunotherapy

Another application is the ex vivo fusion of human dendritic cells and human tumor cells removed from a patient to produce a personal immunotherapeutic vaccine against the tumor. A significant number of experiments were conducted at the Arizona Cancer Center. The results of this research were published in Trevor, et al, 2004, *Cancer Immunol Immunother*, 53(8): 705-714.

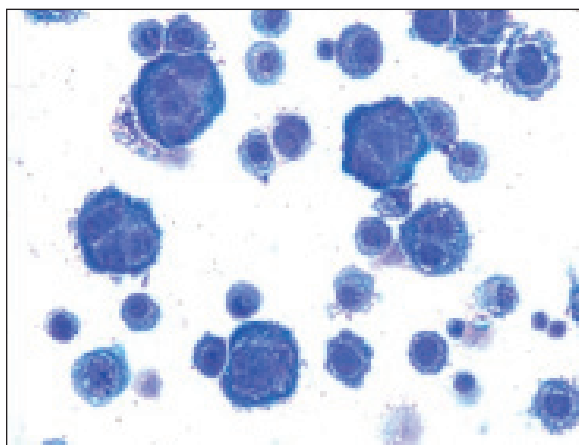


Figure 2: Arizona Cancer Center Fusion Example

Figure 2 shows an electrofusion of dendritic cells with A549 human lung carcinoma cells. Fusion efficiencies of 10% were obtained. Cells were fused at 8 million cells/ml. With that concentration, in a full 9 ml electrofusion chamber, up to 7.2 million therapeutic hybrid cells can be produced. Data provided courtesy of Dr. Katrina Trevor, Arizona Cancer Center.

Figure 3 was produced in the laboratory by fusing an immune system cell and a tumor cell. The image was taken 45 minutes after electrofusion.



Figure 3: Electrofusion Example

Nuclear Transfer

Nuclear transfer is the transfer of a nucleus from one cell to the cytoplasm of another cell. The donor cell can be either from an embryo or from somatic cells. The recipient cell is usually an enucleated oocyte. The process uses electrofusion in the final steps of nuclear transfer. First a recipient oocyte is enucleated. Second, a nucleus from another cell is inserted in the recipient egg under the zona pellucida and outside of the cell membrane. Finally, electrofusion is used to fuse the nucleus with the recipient cell. The electrofusion step also serves to activate the cell. An image furnished by an unidentified Chinese researcher is presented in Figure 4.

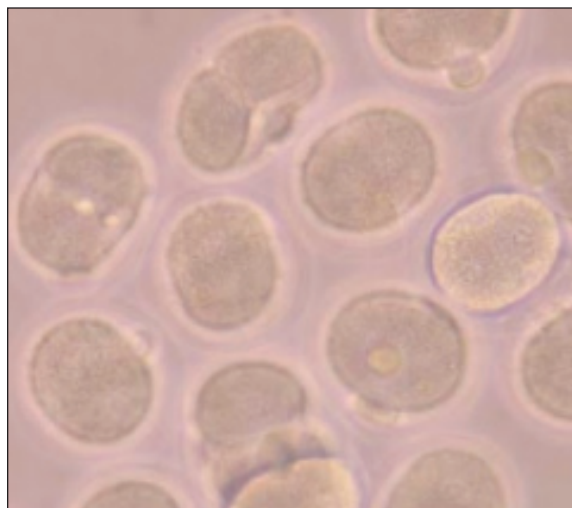


Figure 4: Nuclear Transfer

Cell Electrofusion Tutorial

Cell Fusion Methods

Cell fusion is the formation of a single hybrid cell containing the nuclei and cytoplasm from more than one cell. It is accomplished by:

- **Bringing cells together so that they touch**
- **Compressing the cells to increase contact area**
- **Disrupting the cell membrane**
- **Allowing cell membranes to fuse and providing a good environment for growth of cells**

There are several methods to accomplish these steps. The following table summarizes some of these:

Contact	Fusion
Centrifuge	PEG
Tissue Culture	Virus
Electrical	Electrical
Centrifuge	Electrical
Vacuum	Electrical

Any approach must overcome the natural negative surface charge on a cell. The net charge on a cell as a whole is neutral.

Of these, electrical methods to bring cells into contact and electrical methods to fuse cells are the methods used by the ECM 2001+ System. These also tend to be the most efficient methods.

There are three distinct steps in cell fusion. Each will be discussed in the sections indicated:

1. Pre-fusion process cell processing
2. Electrofusion
3. Post fusion process cell processing

Electrofusion

The process of electrofusion has three steps

1. Cell alignment and compression
2. Fusion
3. Stabilization

The first step is to align the cells and then compress using special electric fields. The ECM 2001+ System uses an alignment and compression technique called dielectrophoresis. The process of manipulating matter using electric fields has been discussed for hundreds of years. The process of using non-uniform electric fields has been used for a number of applications in chemistry, physics, biology and engineering for several decades (Pohl, Dielectrophoresis, Cambridge University Press, 1978). Cell electrofusion was described by Zimmerman in a 1982 paper (Zimmerman and Vienken, *J. Membrane Biology*, 67, 165-182, 1982).

Cell Alignment (Dielectrophoresis)

The first step in electrofusion is to bring the cells together and compress using special electric fields. This is a critical and complex step. Cells have a net zero charge but do have a local negative charge on the surface which acts as a repelling force. The ECM 2001+ System uses an alignment technique called dielectrophoresis that applies a force to overcome the surface charge. From basic physics we know that the force on a charged particle in an electric field is:

$$FORCE = ELECTRIC FIELD \times CHARGE$$

Therefore if a cell, which has a net charge of zero, is placed between two parallel flat electrodes which produce a uniform field, the force applied to the cell is zero. However, there are charges inside the cell such as salt ions. A force is applied to these charges and they will move until stopped, in this case by the cell membrane (an insulator) creating a dipole inside the cell. In a uniform field the force applied is equal and opposite and the cell will not move. However, if a non-uniform field is used the forces are not equal and opposite and the cell will move in the direction of the highest field intensity (see Figure 5).

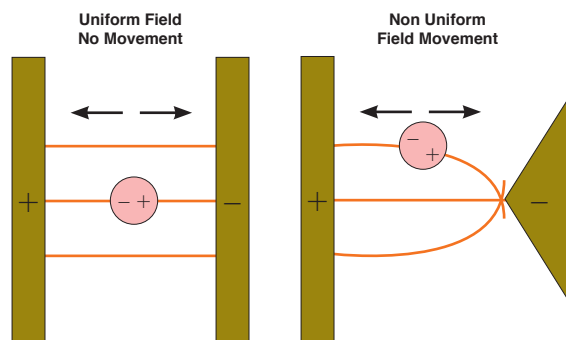


Figure 5: Uniform and Non-Uniform Fields

If there are a number of cells in the non-uniform field, then the cells will line up due to the attraction of unlike charges as shown in Figure 6.

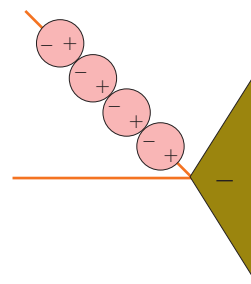


Figure 6: Cell Alignment

Cell Electrofusion Tutorial

Cell Alignment (Dielectrophoresis) (continued)

The actual force applied is a complex formula, see Pohl, Chapter 4:

$$F_{dep} = r^3 [2\pi \epsilon_{medium} K(\epsilon, \sigma, \omega, r)] \nabla E^2$$

The larger the force the faster the cells will move. Although the equation is complex much can be learned by looking at the terms in the equation.

r_c is the radius of the cell

The equation says that the force is proportional to the radius of the cell cubed. As a practical matter when the radius of the cell is below 3 or 4 micrometers, the force falls below that of other forces and the process becomes ineffective. Thus attempting to electrofuse bacteria is not a productive endeavor. This parameter is also of practical interest when attempting to fuse a large cell with a smaller cell. The forces applied to the large cells are larger causing them to move faster.

THE CELL RADIUS IS NOT UNDER USER CONTROL

ϵ_{medium} is the permittivity of the medium

Permittivity, also called electric permittivity (ϵ), is a constant of proportionality that exists between electric displacement and electric field intensity. This constant is equal to approximately 8.85×10^{-12} farads per meter (F/m) in free space (a vacuum). In other materials it can be much different, often substantially greater than the free-space value, which is symbolized ϵ_0 . The medium used in electrofusion is close to high purity water which has a permittivity of $80 \times \epsilon_0$. The permittivity of BTX Cytofusion medium used in the electrofusion process is about $78.5 \times \epsilon_0$. This term is constant over all conditions specified in this User Manual.

THE PERMITTIVITY IS NOT UNDER USER CONTROL

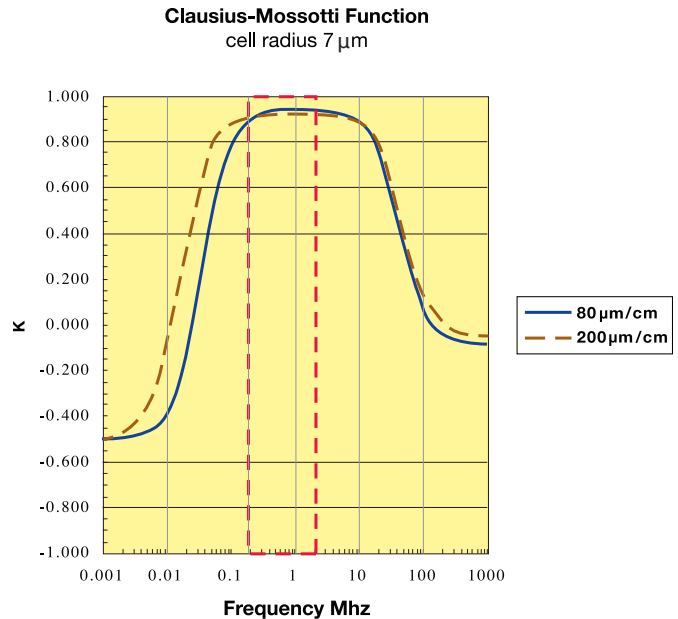
$K(\epsilon, \sigma, \omega, r)$

This term is called the Clausius-Mossotti function. This function represents the time constants of the various ions, medium in the cytoplasm, medium external to the cell, characteristics of the cell membrane and the cell radius. The term $\omega = 2\pi f$ where f is the frequency of the applied alignment waveform.

A computer model of this formula is presented in Figure 7.

K IS NOT UNDER USER CONTROL

A cell radius of $7 \mu\text{m}$ is used in the model which is typical. The model shows, that within typical conductivities of 80 to $200 \mu\text{S/cm}$, the Clausius-Mossotti function is generally in the +0.90 to +0.95 range as long as waveform frequencies are kept within the range of 0.2 to 2 MHz (red rectangle). The ECM 2001+ System, including waveform and medium, was designed to operate in this range.



∇E^2 is the characteristic of the chamber used

The character is the mathematical operator to differentiate with respect to spatial volume. It is important to note that the E is the electric field vector and the electric field intensity is squared. Thus the force on the cell is always in one direction; the force does not reverse direction when a sine wave voltage waveform goes from plus to minus. If the electric field is uniform then:

$$\nabla E^2 = 0$$

Thus with uniform electric fields, such as those produced in a cuvette (parallel plate), the force is zero. There have been publications claiming to have fused cells in a cuvette.

Cell Electrofusion Tutorial

Cell Alignment (Dielectrophoresis) (continued)

The geometry of the chamber and the cell radius are the two major elements that determine the force on the cells. Non-uniform fields can be produced in a number of ways: by two parallel wires, by a wire and a plate, etc. The optimum chamber is two coaxial cylinders called a coaxial chamber. The mathematical model of a coaxial chamber is given by the following (see Pohl, Chapter 7). Chamber dimension definitions are given in Figure 8:

$$V = V_o \frac{\ln [r_g / b]}{\ln [a / b]} \quad V \text{ varies with } r_g \text{ the radial position from } a \text{ to } b \text{ in the chamber gap}$$

$$E_r = \frac{V_o}{r_g \ln [a / b]} \quad E \text{ field varies with } r_g \text{ and is non-linear}$$

$$\nabla E^2 = \frac{-2 V_o^2}{r_g^3 \ln [a / b]^2} \quad \nabla E^2 \text{ varies with } r_g, \text{ and force is}$$

- V_o = The voltage applied from the inner electrode to the outer electrode
 a = The radius of the outside of inner electrode
 b = The radius of the inside of the outer electrode
 a / b = Always less than 1
 r_g = The variable radial distance in between a and b .

As a / b gets small, the gap gets large and ∇E_2 gets small and V_o must increase to compensate. As a / b approaches 1 the gap gets smaller and ∇E_2 gets large and less voltage is required. However, as the gap decreases so does the volume. Increasing the chamber height to hold volume constant has practical limits. It becomes more difficult to maintain a homogeneous cell suspension. A simulation of ∇E_2 and the complete force equation and has been used to define the optimum radius of the inner electrode and the outer electrode in the BTX Fusion chamber to provide the optimum combination of parameters to:

1. Have a quasi-uniform force on the cells in the gap.
2. Maximize volume.
3. Minimize voltage required.

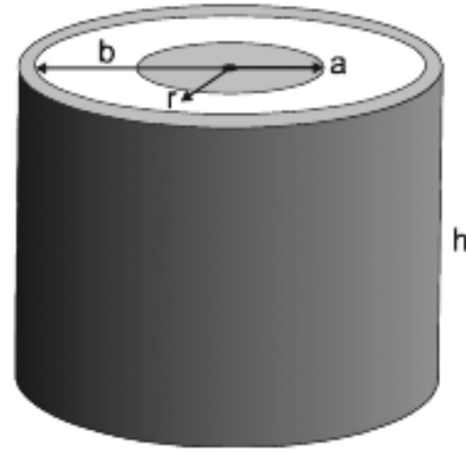


Figure 8: Chamber Definitions

The following is an equivalent presentation:

$$\nabla E^2 = V_o^2 * \frac{-2}{r_g^3 \ln [a / b]^2} \quad V \text{ varies with } r_g \text{ the radial position from } a \text{ to } b \text{ in the chamber gap}$$

This shows the ∇E^2 term has two distinct terms, the first is the amplitude of the AC waveform the second is the geometric

Cell Electrofusion Tutorial

Cell Alignment (Dielectrophoresis) (continued)

Chamber Geometry

The chamber geometry is fixed by the BTX design. A significant amount of effort was expended to determine an “optimum” dimension. The dimensions of the fusion chambers are:

Parameter	Optimization Chamber	Batch Production Chamber
Volume	2 ml	9 ml
Outer Inside Radius (b)	22.86 mm	22.86 mm
Inner Outside Radius (a)	19.05 mm	19.05 mm
Gap	3.81 mm	3.81 mm
Well Height	5 mm	18 mm
a/b	0.8333	0.8333

The two chambers are electrically equivalent. As shown in the table above height is not a factor, only the radius of the inner and outer electrodes. An image of cell alignment in the 2 ml optimization chamber is presented in Figure 9.

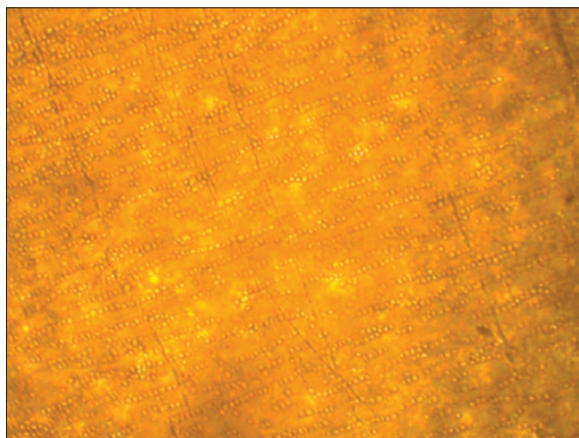


Figure 9: Cell Alignment in Optimization

The direction of the force on the cells in the fusion chamber is from the outer to inner electrode. The field is non-uniform from outer to inner electrode. The force does not change as a function of chamber height which is a very significant advantage over other electrode configurations.

AC Waveform Amplitude

The AC waveform amplitude and duration are the only variables under user control. The user can control force by changing amplitude. In addition, the duration of the AC waveform applies the force for a given period of time which is also under user control. Frequency is available but Figure 7 indicates it has little effect. The graph in Figure 10 presents the approximate force applied to a cell as a function of cell radius (5, 10 and 20 μm) versus AC peak voltage. The AC peak voltage range is that provided by the ECM 2001+ waveform generator. The effect of the cell radius cubed is quite dramatic. This force is on a cell in isolation.

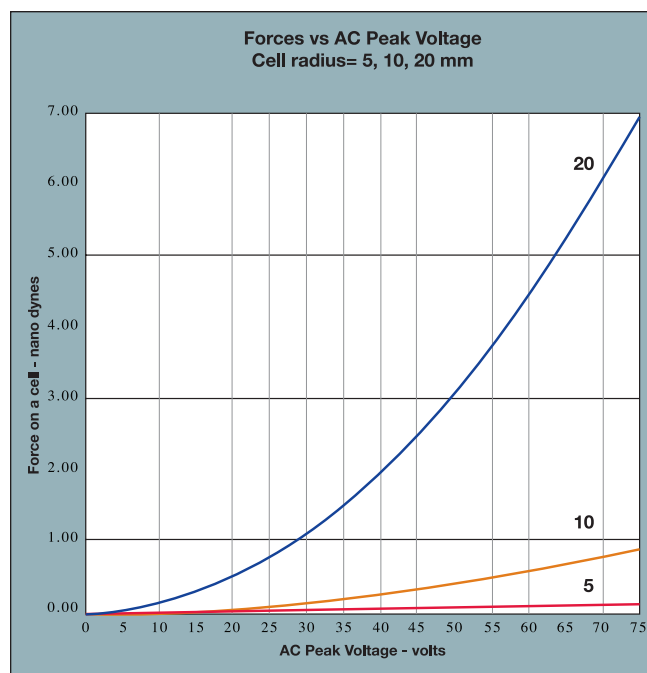


Figure 10: Force on a Cell

In summary, the design of the ECM 2001+ System has been optimized for electrofusion and many of the variables are fixed by system design such as the chamber configuration and the conductivity of the medium. One powerful tool available to the user is the selection of the waveform protocol. The waveform protocol sets the force on the cells for alignment and compression, the pulse to fuse the cells, and the waveform to stabilize the fused cells after the pulse. The next section covers the available waveforms.

Cell Electrofusion Tutorial

Waveforms for Electrofusion

The two most important metrics in an electrofusion system for a given cell radius are the chamber configuration and the voltage applied to the chamber to produce the electric field. In the last section the chamber parameters were discussed. In this section the voltage waveform parameters will be discussed.

There are two types of waveforms used, alternating current (AC) in the form of a sine wave and pulsed direct current (DC). The functions of these waveforms in the electrofusion process are:

Function	Waveform Type	Typical ¹
Cell alignment and compression	AC sine wave ²	Non-linear increasing amplitude
Fusion	DC Pulse	One short pulse
Fusion partner stabilization	AC sine wave ²	Linear decreasing amplitude

Notes:

1. There have been optimized protocols that use waveforms other than those shown as typical, for example see Trevor et. al, 2004.
2. There are many types of AC waveforms, rectangular, square, trapezoid, triangle, and sine wave. ECM 2001+ technology uses sine wave AC waveforms due their smooth amplitude transition.

Cell Alignment and Compression

There are two important parameters, the chamber configuration and the voltage amplitude. The chamber configuration has been optimized and is fixed. However, the voltage parameters can be set by the user. The objective of this step in electrofusion is to align the cells in an optimum manner and then apply the fusion pulse. There are a number of important considerations:

1. A perfect alignment process would alternate the two cell types to be fused. In general that does not occur in any known process. The way the cells align is a statistical event. If the cells all have the same radius and equal numbers of each are mixed, then (see Figure 11):

$$XY = 25\%$$

$$YX = 25\%$$

$$XX = 25\%$$

$$YY = 25\%$$

$$XY + YX = 50\%$$

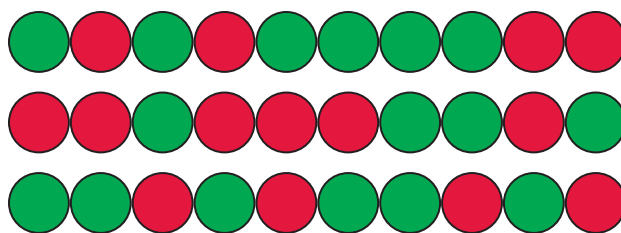


Figure 11: Cell Alignment Sequence

In addition not all cells fuse in pairs; some products have 3, 4 or more nuclei. This can be controlled but not eliminated by optimization of waveform amplitude.

A practical result of the stochastic nature of cell alignment is that the maximum yield is obtained when the percent of each cell population is 50% (shown above). However, when 3 cells of X are present for every one cell of Y (75%:25%), then the following yield is obtained:

$$YX = 19\%$$

$$XY = 19\%$$

$$XX = 56\%$$

$$YY = 6\%$$

$$XY + YX = 38\%$$

Unequal ratios of cells are sometimes used to conserve a rare cell population. However, when this is done, the total yield decreases.

2. The force on the cell during alignment is a function of the cell radius cubed. The radius of the cell varies within the same type and the mean radius of the two cell types to be fused varies. This will affect the number of fusion products produced.
3. Some cell types are much more difficult to fuse than others. This is inherent in the cell characteristics.
4. In the initial alignment process the cells will accelerate when the force is applied. If the acceleration is significant then the cells will move toward the center electrode and bounce off like a billiard ball. It is possible to create such acceleration that the cells begin circulating in a “race track” pattern. High acceleration is very detrimental to good cell alignment and must be avoided.

The principal use of the BTX 2 mL “Optimization” Chamber is waveform protocol optimization. The alignment process must be optimized while observing the cell alignment.

Cell Electrofusion Tutorial

Waveforms for Electrofusion (continued)

5. The initial alignment must be done slowly and deliberately so good alignments can be produced. However if a small force is used to align as required then compression will not occur. This still leaves the function of compression to increase the surface contact between adjacent cells to increase the probability of a successful fusion pair. After alignment the cells will be in contact tangentially but not compressed. The solution to this problem is to use a non-linear AC amplitude. The first part of the AC sine wave will be low amplitude and then immediately increase the amplitude to compress while the cells are in contact. The alignment chains are much less likely to break up at this point since they have cells touching on each side. The ECM 2001+ voltage waveform generator will produce a number of these types of waveforms. These are described below.
6. Care has to be taken with the amount of time the AC sine wave is applied. If left on too long the current will heat up the medium like the heating element in a coffee maker. Heat causes convection currents which in turn causes the chains to break apart.

AC Sine Wave Waveforms

The definition of AC sine wave amplitude and frequency is shown in Figure 12. Electrical engineers use several definitions for amplitude. As shown half of the sine wave is positive and half is negative. The concept of average value is meaningless since that is zero. There are three common definitions in use:

- **Peak Voltage** is the amplitude from zero to the peak positive or negative value
- **Peak-to-Peak Voltage** is the value from the negative peak to the positive peak
- **rms Voltage** is square root of the mean value of the sine wave squared; this is the power in the waveform (root mean squared)

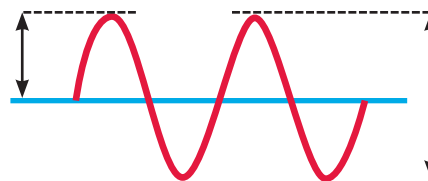


Figure 12: Sine wave Definition

The ECM 2001+ voltage waveform generator uses the definition of Peak Voltage or Vpk. The table below shows the relationship between the various definitions. Also included is the standard USA mains voltage. Note: the 120 V (240 V in many other countries) rating on appliances is actually rms. The bottom of the table shows that peak-to-peak at the wall socket (power mains) is actually 340 volts.

Vpk-to-pk	Vpk	Vrms
10	5	3.5
20	10	7.1
30	15	10.6
40	20	14.1
50	25	17.7
60	30	21.2
70	35	24.7
80	40	28.3
90	45	31.8
100	50	35.4
110	55	38.9
120	60	42.4
130	65	46.0
140	70	49.5
150	75	53.0

340 (US mains)	170	120
680 (EU mains)	340	240

Cell Electrofusion Tutorial

Waveforms for Electrofusion (continued)

The voltage range of the ECM 2001+ voltage waveform generator can be set from 5 to 75 volts peak in 5 volts-peak steps using several algorithms. These algorithms and their names are presented in Figure 13.

Constant Amplitude

This function is the classic electrofusion waveform and has appeared on a number of vendor's systems. Generally the amplitude is set and the duration is set. This function is available on the BTX system.

Linear Ramp Amplitude (set by Ramp $k = 1$)

This function appeared on the original Zimmerman system a number of years ago. That system has not been sold for many years. The linear ramp can be set to increase or decrease at a constant rate volts per second by setting the start voltage, the stop voltage and the duration. This function is also available on the BTX system.

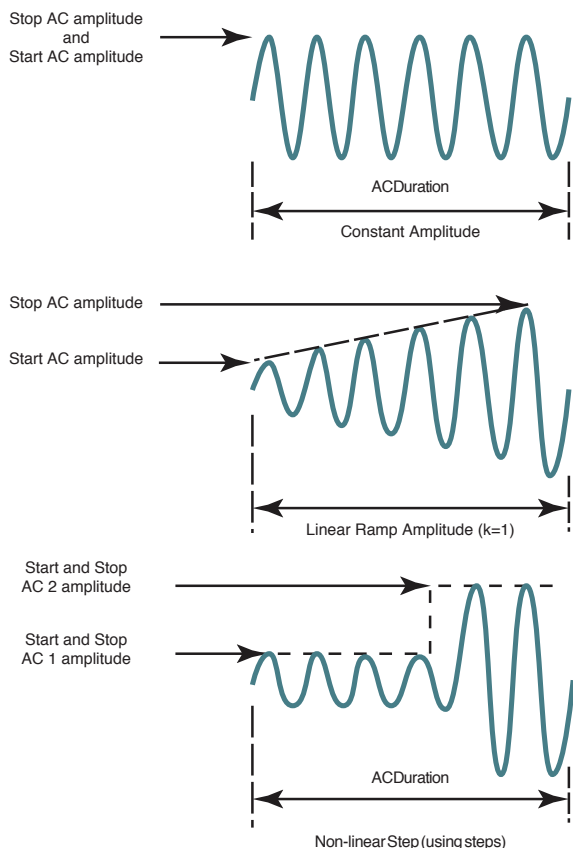


Figure 13: AC Waveform Algorithms

Electrofusion Pulse

The function of this waveform is to form pathways in the cell membranes to allow the cytoplasm to mix and the cells to fuse. One or more high electric field pulses are needed. This process is electroporation and is illustrated in Figure 14. An electric field is applied that is high enough to induce a voltage across the cell membrane that induces pore formation. The minimum transmembrane voltage is approximately one volt. This requires

applying electric fields of hundreds of volts/cm to achieve the at least one volt across the cell membrane. Small diameter cells require higher electric fields than do large diameter cells.

Theoretically, the formation of a pore in one cell increases the likelihood that a pore will form in an adjacent cell membrane in the same location as the original pore. This is because pores in cell membranes conduct electricity through the pore.

Whatever the actual mechanism of formation, pores in cells whose membranes are touching can re-seal in one of two ways. One is that the pore will simply close in the cell membrane. Another is that adjacent pores in the membranes of two touching cells will fuse together, combining the cell membranes of two cells.

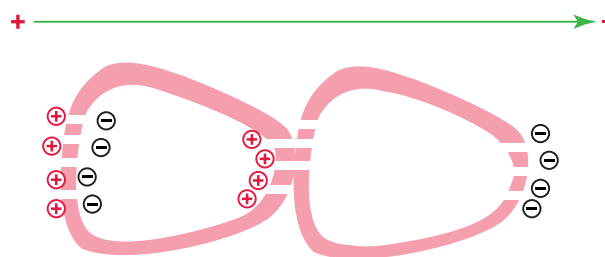


Figure 14: Electric Field Pulse Forms

Post Fusion Pulse AC Sine Wave

The objective of this function is to hold the cells in place that have been fused together, to allow them to mature. Immediately stopping the AC sinewave causes the cells to break apart by thermal motion. Experience has shown that a simple decreasing Linear Ramp Amplitude ($k = 1$) has proven to be the most effective.

Parameter Range of ECM 2001+ Voltage Waveform Generator

The following parameters may be programmed in the waveform generator.

AC Before Fusion Pulse and AC After Fusion Pulse	Amplitude	5 to 75 V peak, 5 volts steps
	Amplitude Function	Constant Linear increase or decrease ($k=1$)
	Frequency	0.2 to 2.0 MHz in 0.1 MHz steps
	Duration	0 to 99 seconds in 1 second steps Set zero inhibits AC
Fusion Pulse	Amplitude	5 to 500 V in 1V steps or 505 to 3000 V in 5 V steps
	Width	If V less than or equal to 500V, 10 microseconds to 999 ms If V >500 V, 10 to 600 microseconds
	Interval	0.1 to 10.0 seconds in 0.01 second steps
	Number	0 to 99 in steps of 1, 0 inhibits pulse

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 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, 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.

voltage = 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:

$$TMV \ 3/2 = E \ r \ | \cos \alpha|$$

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 μ 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).

Electroporation Overview

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 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, 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 electroporation in in vivo and ex vivo tissues, embryos, and plant protoplast applications yield better results in comparison to an exponential decay wave. This is the waveform used by the ECM 2001+ system.

Exponential Decay Wave Pulse

Exponential decay waves generate an electrical pulse by allowing a capacitor to completely discharge. When 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. This waveform is not available on the ECM 2001+ system. This waveform is used by either the BTX ECM 630 or BTX Gemini systems.

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 to 24 kV/cm

Mammalian: 0.25 to 3 kV/cm

Plant: 3 to 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 500 V would provide a field strength of 1.25 kV/cm. If instead of a 4mm 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 1mm gap cuvette the calculation would be: $1.25 \text{ kV} \times 0.1 \text{ cm} = 0.125 \text{ kV}$ 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 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 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 1/3 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

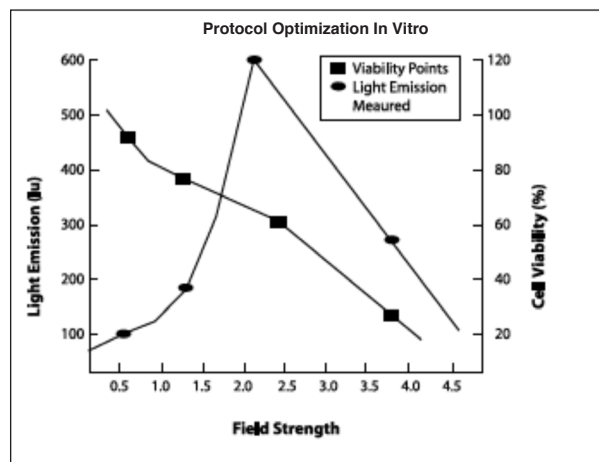
The buffers used for electroporation can vary depending on the cell type. Many applications use highly conductive buffers such as PBS (Phosphate Buffered Saline <30 ohms) and HBSS (Hepes Buffer <30 ohms) or standard culture media which may contain serum. Other recommended buffers are hypoosmolar buffers 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 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.

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 5–20 g/ml for most cell types; however in some instances increasing the DNA concentration as high as 50 g/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.



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 with yield high transfection efficiencies and great cell survival rates.

In Vivo, In Utero, In Ovo

Square waves 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.

Ordering Information

Item No.	Description	Qty.
ECM 2001+ Systems		
45-2045	ECM 2001+ Cell Fusion System Complete Includes: ECM 2001+ Generator, Microslides (0.5 mm Gap, 3.2 mm Gap), Meander Fusion Chamber, Flat Electrode / Divergent Field, Electrode Adapter, Connection Cable, Safety Stand 630B, Cuvettes 1 mm, 2 mm, 4 mm pkg. of 30 (10 each) and Cuvette Rack	1 ea
45-2046	ECM 2001+ Electroporation System Complete, for Cuvettes Includes: ECM 2001+ Generator, 630B Safety Stand, Cuvettes 1 mm, 2 mm, 4 mm pkg. of 30 (10 each) and Cuvette Rack	1 ea
45-2047	ECM 2001+ Embryo Manipulation System Includes: ECM 2001+ Generator, Microslides, round wire (0.5 mm gap, 1.0 mm gap), rectangular wire (3.2 mm gap), Micrograbber adapter cables	1 ea
45-2048	ECM 2001+ Hybridoma Production System Includes: ECM 2001+, Coaxial chambers (2 ml optimization, 9 ml production), High voltage cable, micrograbber adapters, BTX Cytofusion Medium C (500 ml)	1 ea
45-2049	ECM 2001+ Generator Only Includes: ECM 2001+ Generator only	1 ea
45-2050	ECM 2001+ System Complete, for Cuvettes with Monitoring Includes: ECM 2001 Generator, 630B Safety Stand, Cuvettes 1 mm, 2 mm, 4 mm Pkg/30 (10 each), Cuvette Rack 660, Enhancer 3000 Probe, ENHANCER Interface Box, Oscilloscope and Cables	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
45-0135	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
45-0136	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
Reagents		
47-0001	BTXpress Cytofusion Medium C, 500 ml volume	1 Bottle
47-0003	BTXpress Cytoporation Low Conductivity Medium T4, 500 ml volume	1 Bottle
Accessories		
47-0030	2 ml Coaxial Chamber for optimization	1 ea
47-0020	9 ml Coaxial Chamber for production	1 ea
45-0108	Flat Electrode for Cell Fusion	1 ea
45-0103	Glass Microslides with round wire electrodes, 0.5 mm gap, 20 µl, Pkg/10	1 pkg

Item No.	Description	Qty.
Accessories		
45-0104	Glass Microslides with round wire electrodes, 1.0 mm gap, 40 µl, Pkg/10	1 Pkg
45-0105	Glass Microslides with rectangular electrodes, 3.2 mm gap, 650 µl, Pkg/1	1 Pkg
45-0106	Glass Microslides with rectangular electrodes, 10 mm gap, 2.0 ml, Pkg/1	1 Pkg
45-0107	Meander Fusion Chamber	1 ea
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/50 (Requires Safety Stand 45-0207)	1 Pkg
45-0109	Flatpack Chambers, 1.83 mm gap, 1.5 ml volume, Pkg/50 (Requires Safety Stand 45-0207)	1 Pkg
47-0206	Flatpack Chambers, 4 mm gap, 10 ml volume, Pkg/10 (Requires Safety Stand 45-0207)	1 Pkg
47-0208	Safety Stand, Adjustable Gap (for use with Cuvettes and Flatpacks)	1 ea
45-0122	Genepaddles, 3 x 5 mm (need 45-0203 holder and 45-0216 cables)	1 ea
45-0169	Genepaddles Kit, 3 x 5 mm (includes 45-0203 holder and 45-0216 cables)	1 ea
45-0123	Genepaddles, 5 x 7 mm (need 45-0203 holder and 45-0216 cables)	1 ea
45-0170	Genepaddles Kit, 5 x 7 mm (includes 45-0203 holder and 45-0216 cables)	1 ea
45-0203	Genetrode/Genepaddle Holder	1 ea
45-0117	Genetrodes, 1 mm L-Shape (GOLD TIP)	1 ea
45-0164	Genetrodes, 1 mm L-Shape (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 ea
45-0116	Genetrodes, 3 mm L-Shape (GOLD TIP)	1 ea
45-0163	Genetrodes, 3 mm L-Shape (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 ea
45-0115	Genetrodes, 5 mm L-Shape (GOLD TIP)	1 ea
45-0162	Genetrodes Kit, 5 mm L-Shape (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 ea
45-0113	Genetrodes, 5 mm Straight (GOLD TIP)	1 ea
45-0160	Genetrodes Kit, 5 mm Straight (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 ea
45-0114	Genetrodes, 10 mm Straight (GOLD TIP)	1 ea
45-0161	Genetrodes Kit, 10 mm Straight (GOLD TIP) with 45-0203 holder and 45-0216 cables	1 ea
45-0100	Petri Dish Electrode, adherent electrode for 10 cm dishes	1 ea
45-0504	Petri Dish Platinum Electrode Chamber, 5 mm gap	1 ea
45-0506	Petri Dish Platinum Electrode Chamber, 15 mm gap	1 ea
45-0513	Petri Dish Tissue Chamber Kit, 15 mm gap with cables	1 ea

Ordering Information

Item No.	Description	Qty.
Accessories		
45-0505	Petri Dish Platinum Electrode for Tissues Chamber Kit, 5 mm gap	1 ea
45-0507	Petri Dish Platinum Electrode for Tissues Chamber Kit, 15 mm gap	1 ea
45-0491	Petri Dish Platinum Electrode Chamber, 7 mm, negative	1 ea
45-0492	Platinum Electrode Wand, 7 mm, positive	1 ea
45-0490	Petri Dish Platinum Electrode for Tissue Slices Chamber Kit, 7 mm	1 kit
45-0501	Petri Dish Platinum Electrode Chamber, 10 mm, negative	1 ea
45-0502	Platinum Electrode Wand, 10 mm, positive	1 ea
45-0500	Petri Dish Platinum Electrode for Tissue Slices Chamber Kit, 10 mm	1 kit
45-0130	Petri Pulser	1 ea
45-0525	Platinum Tweezertrode Kit, 1 mm Flat (includes 45-0204 cable)	1 ea
45-0486	Platinum Tweezertrode, 1 mm Diameter (includes 45-0204 cables)	1 ea
45-0524	Platinum Tweezertrode, 1 mm Flat	1 ea
45-0487	Platinum Tweezertrode, 3 mm Diameter (includes 45-0204 cable)	1 ea
45-0489	Platinum Tweezertrode, 5 mm Diameter (includes 45-0204 cables)	1 ea
45-0488	Platinum Tweezertrode, 7 mm Diameter (includes 45-0204 cables)	1 ea
45-0118	Stainless Tweezertrode Electrode, 7 mm Diameter	1 ea
45-0165	Stainless Tweezertrode Kit, 7 mm (includes 45-0204 cable)	1 ea
45-0119	Stainless Tweezertrode Electrode, 10 mm Diameter	1 ea
45-0166	Stainless Tweezertrode Kit, 10 mm (includes 45-0204 cable)	1 ea
45-0493	Triple Electrode Tweezertrode, 3 mm	1 ea
45-0494	Triple Electrode Tweezertrode, 5 mm	1 ea

Item No.	Description	Qty.
Cables & Adapters		
45-0168	2-Needle Array Kit, 5mm, Pkg/6, with Handle	1 ea
45-0167	2-Needle Array Kit, 10mm, Pkg/6, with Handle	1 ea
45-0101	Caliper Electrode, 1 x 1 cm, Brass, Transdermal Delivery Applications	1 ea
45-0102	Caliper Electrode, 2 x 2 cm, Stainless Steel, Electrochemotherapy Applications	1 ea
45-0087	ECM 2001+ Adaptor Set, Micrograbber to Banana Plug Cables	1 ea
45-0216	ECM 2001+ Micrograbber to Banana Plug Connection Cables, Red and Black, 10 ft (for use with microslides, genetropes and tissue petri dish)	1 ea
45-0083	ECM 2001+ Coaxial to Banana Plug Cables, Red and Black, 10 ft	1 ea
45-0088	ECM 2001+ Female/Female Adapter Set for Banana Plug Cables (Use with 45-0216)	1 ea
45-0217	ECM 2001+ Banana to Banana Cables, Red and Black, 10 ft (for use with 45-0108 Flat Electrode)	1 ea
Accessories		
45-0168	2-Needle Array Kit, 5 mm	Pkg/6
45-0167	2-Needle Array Kit, 10 mm	Pkg/6
45-0530	Adherent Cell Electrode, 5 mm Gap	1 ea
45-0531	Adherent Cell Electrode Kit, 5 mm Gap	1 ea
45-0101	Caliper Electrode, 1 x 1 cm, Brass, Transdermal Delivery Applications	1 kit
45-0102	Caliper Electrode, 2 x 2 cm, Brass, Electrochemotherapy Applications	1 ea
45-2030	Footswitch	1 ea
45-2057	ECM 2001+ High Voltage Output Cable, 5 ft length	1 ea
45-2058	ECM 2001+ High Voltage Output Cable, 10 ft length	1 ea

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