

# AgilePulse™ MAX

## Large Volume Transfection System

USER'S MANUAL



**BTX**®

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

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# A Message from BTX

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## **Thank you for investing in an AgilePulse system.**

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

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

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

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

### **BTX**

84 October Hill Road  
Holliston, MA 01746 USA

Toll Free: 1-800-272-2775

International Callers: 508-893-8999

Fax: 508-429-5732

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

Email: [support@hbiosci.com](mailto:support@hbiosci.com)

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



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

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# Warranty Information

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## RESEARCH ONLY

### BTX

84 October Hill Rd  
Holliston, MA 01746, USA

Phone: 1-508-893-8999

Fax: 1-800-429-5732

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

## Warranty

BTX warranties AgilePulse systems for a period of two years from the date of purchase. At its option, BTX will repair or replace the product if it is found to be defective as to workmanship or materials. This warranty does not extend to any device 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 BTX AgilePulse system 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 AgilePulse instrument. If a defect arises within the warranty period, promptly contact BTX, 84 October Hill Road, Building 7, Holliston, Massachusetts, USA 01746-1388 using our toll free number 1-800-272-2775 (US Only) or 508-893-8999 (E-mail: [support@hbiosci.com](mailto:support@hbiosci.com)). Goods will not be accepted for return unless an RMA (Returned Materials Authorization) number has been issued by our customer service department. The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device. This warranty gives you specific rights, and you may also have other rights, which vary from state to state.

## Out of Warranty Service

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

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

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

## Repair Facilities and Parts

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

## Caution Notice

The BTX AgilePulse systems are intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) and safety intended for laboratory equipment applications.

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

# Safety Information

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

## To Prevent Hazard or Injury

### Emergency Stop

If a problem occurs during a run, push the STOP/RESET button on the front panel.

### Use Proper Line Cord

Use only the specified line cord for this product and make sure line cord is certified for country of use. The operating voltage range for the AgilePulse Series is 100 to 240 VAC, 50/60 Hz.

### Ground the Product

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

### Make Proper Connections

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

### Observe All Terminal Ratings

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

### Use Proper Fuse

Use only specified fuses with product.

### Avoid Exposed Circuitry

Do not touch any electronic circuitry inside of the product.

### Do Not Operate with Suspected Failures

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

### Orient the Equipment Properly

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

### Place Product in Proper Environment

Review the operating manual for guidelines for proper operating environments.

### Observe All Warning Labels on Product

Read all labels on product to ensure proper usage.

## High Voltage Risk

These instruments contain a high voltage power supply adjustable to 1,200 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.
- Do not under any circumstances turn the power switch off while the system is pulsing; if an emergency occurs push the emergency button on the front panel

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

## Caution Notice

The BTX AgilePulse Systems are intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) intended for laboratory equipment applications 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

# AgilePulse™ MAX System Components & Set-Up

## Introduction

The AgilePulse™ MAX system will transfect a large volume of cells in a single electric field application. The system is designed to be easy to set-up and to operate in a research environment.

The AgilePulse MAX System is composed of five subsystems:

- Touch screen controlled Waveform Generator
- 5 ml large volume chambers (Cable, Base and Chamber Electrode)
- Safety Stand (used for cuvette and flatpack electroporation)
- Cytoporation® Medium
- User Manual

**These components were all designed to operate as an integrated system. Using other components may damage the system and will certainly provide degraded performance and invalidate void the warranty.**

## The AgilePulse™ MAX Waveform Generator

The Waveform Generator is a computer with high voltage pulsing circuits (Figure 1). The computer runs Windows® 10 and is operated via a touch screen on the front panel. The computer has a power connection on the back panel and a power switch, emergency stop switch, electrode connector and two USB ports on the front panel. User inputs and outputs may be handled through the touch screen or via a keyboard connected to one of the USB ports.

The system is controlled by a microcontroller. The microcontroller accepts user inputs from the touch screen and produces the pulse waveforms. A second microcontroller is used as an independent audit of the waveforms produced. If the independent audit function detects a deviation from the pulse protocol, the system immediately terminates pulsing. The system also digitizes the output of the pulse voltage and pulse current monitors and calculates the resistance of the volume processed. This is a very significant quality control function that indicates the electric fields are being applied correctly.

After the cells in solution are transfected, a complete log of all parameters is saved in internal memory. If a USB memory key is inserted, the data is also written to the key. The key may also be inserted at a later time and a particular run can be saved to the key.



Figure 1: Waveform Generator

# AgilePulse™ MAX System Components & Set-Up

## Front Panel

The following functions are on the front panel: the Mains/Line power switch (illuminated when on), the Emergency Stop button switch, two USB ports, the electrode connector, and the User Touch Screen (Figure 2).

One USB port is used for a Memory Key. The data logs that result from a transfection run are automatically stored on the key if the key is inserted.

The Emergency Stop button switch is used to stop system operation in an emergency situation. This switch immediately stops all pulsing and turns off the high voltage power supply.



Figure 2: Waveform Generator Front Panel



Caution

**The Mains/Line power switch should never be turned off in an emergency situation while pulsing is in progress.**

## Back Panel

The back panel has two connectors: an IEC Power Entry module and an Ethernet connection. The Ethernet connection is currently not functional, but is built in for expansion of future versions.

## Large Volume Chamber & Base

The Large Volume Chamber consists of two flat and parallel plates with 6 mm spacing. There are two Luer connectors. The lower Luer port is for connecting a syringe to load cell suspension sample into chamber. This lower Luer port also has a stopcock valve to allow for opening and closing of the port during loading and removal of sample. The second Luer connector located on the top of the chamber is fitted with an air filter and acts as a vent during electroportion. The cells to be transfected are suspended in Cytoporation® Medium, mixed, and loaded in the chamber via the lower Luer lock connector using a syringe. Following cell loading into the chamber, close the valve and remove syringe. The chamber is then placed into the base. The chamber can only be inserted into the base in one direction. The end with the plastic protrusion must face the base to be inserted properly. (See Figure 3)

The chamber can be used with a minimum of 2 mL and a maximum of 5 mL. The chamber should be used one time unless a validated cleaning procedure is established, documented and allowed by the protocol.



Figure 3: Chamber and Base

## Safety Stand for Flatpack

The Safety Stand for Flatpack can accept 4 mm gap, 10 ml electroportation flatpack chambers, or standard 1, 2, or 4 mm gap electroportation cuvettes. Its unique design ensures that no current can be delivered unless the lid is closed therefore protecting the user from high voltages.

To use, the safety stand shield is opened and the flatpack chamber or electroportation cuvette is placed between the metal contacts. The distance between the contacts is adjusted using the black roller. (Note: The adjustment will remain the same after the first time. It is not necessary to readjust the roller with every cuvette; however check contact periodically to ensure good current flow). The safety stand shield is then closed during the electroportation pulsing procedure (See Figure 4).



Figure 4: Safety Stand for Flatpack



# AgilePulse™ MAX System Components & Set-Up



Figure 5: Cytoporation® Medium

## Cytoporation® Medium

BTX provides low conductivity media that are especially formulated for large volume electroporation. There are currently two formulas available with different conductivities (See Figure 5):

Parameter	Specification
Sterility	Sterile filtered
pH	7.2
Conductivity	0.08 mS/cm (Medium T) 3.45 mS/cm (Medium T4)
Endotoxin	< 0.2 IU/ml
Osmolarity	270 to 290 milliosmoles
Storage	4 to 8 °C After Opening
Packaging	500 ml bottle

See the AgilePulse MAX Tutorial section for more details about conductivity and large volume transfection.

## Setting Up the System

The system set up and testing procedure is as follows:

1. Unpack the contents of the shipping box.
2. Check for obvious signs of exterior damage. If damage is noted, contact BTX Technical Support before proceeding.
3. Place the Waveform Generator on the top of a sturdy table.
4. Connect the Chamber Base cable into the connector at the bottom right of the front panel.
5. Connect the mains/power cord into the back panel at the bottom right.

## Initial System Test

This section will describe the process to verify mains/line cable has been installed properly and the computer boots up.

1. Connect the power cord to the mains.
2. Turn the rocker switch on the front panel to the “on” position (I).

## What should happen?

The rocker switch, “Power”, should illuminate and the Opening Touch Screen should appear within 10 seconds. If the Waveform Generator power switch fails to illuminate, then return it to the OFF position (O). Verify that the power cord is properly plugged into the wall. Verify the wall socket is functional. It may be necessary to check the fuse. See Maintenance section for instructions, proper replacement of the fuse and the correct fuse type. If a problem persists, call BTX Technical Support for assistance.

If the power is properly applied and the touch screen is illuminated then the following display is visible:

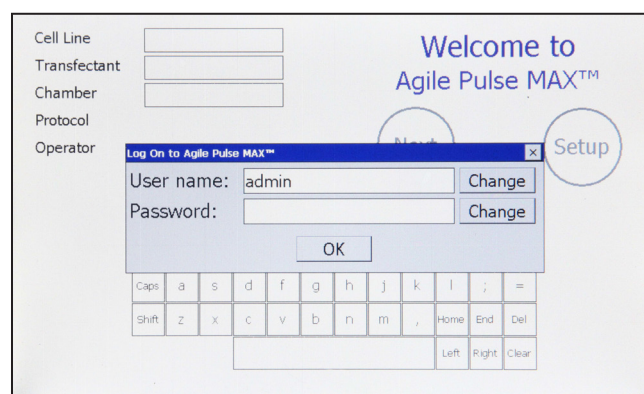


Figure 6: Start Up Screen Display

This completes the set-up and testing of the AgilePulse™ MAX System. If there is a problem, contact Technical Support for assistance.

Phone **508-893-8999**, or toll free **800-272-2775**

Website **www.btxonline.com**

# AgilePulse™ MAX System Operation

This chapter gives a detailed description of the operation of the AgilePulse™ MAX system.

## Waveform Generator

The Waveform Generator is controlled by a touch screen computer. It is recommended that the user touch the screen with the supplied stylus. Field entries can be made with the on-screen virtual keyboard. Alternatively, a computer keyboard may be attached via one of the USB ports.

The process flow is:

- **Login**
- **User Data Entry**
- **Initialize Pulse Sequence**
- **Pulse Delivery**
- **End Process**

## Login Screen — User Identification Input

After power is turned on, a Login Screen is presented that requires the user to enter a user name and password combination. The Login system is designed to prevent unauthorized users from accessing the system and confine authorized users to permissible activities.

Two types of users are defined: system administrator and standard user. There is only one system administrator of the system who has the ability to add/remove pulse waveform protocols, add/remove standard user accounts, and assign pulse waveform protocol access privileges to standard users. The administrator always operates under the “admin” account.

Standard users are intended to be everyday users of the system, who only have the ability to run pre-defined pulse waveform protocols from an access list as determined by the administrator. The administrator may grant a standard user the ability to modify an already defined protocol, but not to add/remove protocols.

AgilePulse™ MAX is factory configured with only the “admin” account available. By default no password is defined so the user can enter the system with parameters matching that of Figure 7 on first login. The password may then be changed from the User account screen in Setup section. As default, the most recently logged in user will be displayed in the User Name field on startup.

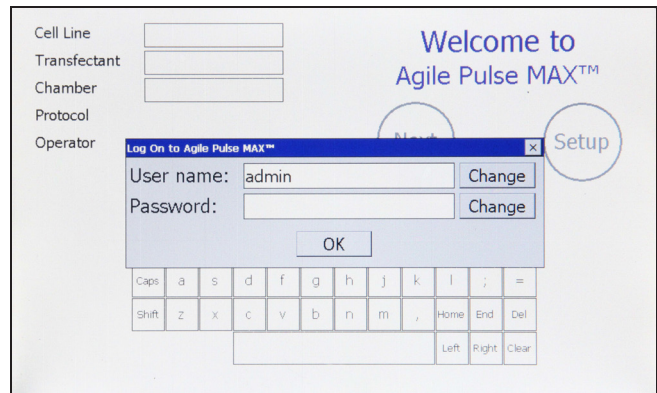


Figure 7: Login Screen

To enter user names and passwords, touch the Change buttons to activate a virtual keyboard.

**User Name:** Enter the user’s name to log in.

**Password:** Enter the password associated with the user account. Password entry is masked with “•” characters to provide increased security.

When the User name and password have been entered, touch OK to log in.



**IMPORTANT! Errors can be generated if power is cycled off and on too quickly. Wait at least five seconds after turning power off before turning power back on.**

# AgilePulse™ MAX System Operation

## Welcome Screen — Sample Identification Input

A Welcome Screen will be presented following successful user log in that contains fields that should be completed before the electroporation process is initiated. Information is entered into each box by a virtual keyboard. Entering data into each field is optional. Fields may be left blank before proceeding to the next screen (See Figure 8).

To change pulse parameters, touch SETUP before advancing to pulse initiation. Pre-programmed protocols are selected from a drop-down menu on the PROTOCOLS screen (see Protocol Setup, page 11). Information entered into the data fields will be retained when returning from SETUP. The data fields are cleared upon completion of pulse delivery.

When all of the fields are filled in and the desired protocol has been selected, pulse delivery is initiated by touching NEXT to advance to the Pulse Delivery Screen.

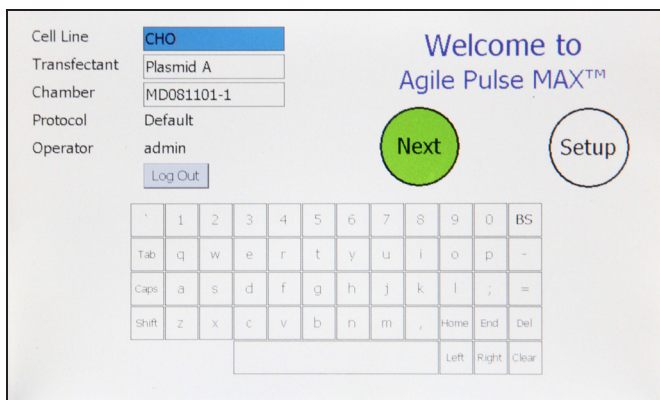


Figure 8: Welcome Screen

## Pulse Delivery Screen: Functions and Operation

The Pulse Delivery Screen is used to control the delivery of electroporation pulses to the sample. The Pulse Delivery Screen also provides visual feedback before, during, and after pulse delivery.

### Procedure for Pulse Delivery:

1. Fill a Large Volume Electroporation Chamber with 2 to 5 ml cell suspension plus polynucleotide (such as plasmid DNA).
2. Insert the Chamber in the Chamber Holder. Do not insert fingers or other objects in the Chamber Holder.
3. Touch the Load button. The system will automatically deliver a short low voltage pulse to estimate the load resistance. If the resistance is too low, the pulse will not be delivered. If the resistance is within acceptable limits, the Ready button will turn yellow.

4. Touch the Ready button. The system will charge the high voltage capacitors to the set voltage. When the system is charged, the Start button will turn yellow.
5. Touch the Start button. The Figure 9: Pulse Delivery Screen – Pre-Pulse system will deliver the set pulse protocol. When delivery is complete, the Done button will turn green and the pulse log will be displayed in the text box.
6. Touch the Done button to return to the Welcome Screen. The identification fields will be reset. Repeat the procedure for each pulse delivery (See Figure 10).



Figure 9: Pulse Delivery Screen — Pre-Pulse

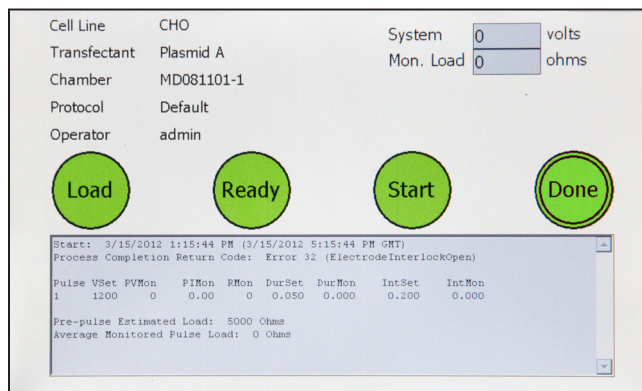


Figure 10: Pulse Delivery Screen — Post-Pulse

# AgilePulse™ MAX System Operation

## User Account Maintenance

The user account maintenance screen is accessed from the Welcome screen:

### Touch SETUP

### Touch USER

User accounts and privileges are managed from this screen. While standard users will only see the Active User section, and thus will only be able to change their password, the system administrator has access to account properties for all standard users.

As an administrator, a new user account can be added by pressing the "Add New User" button. Once the account is created the new user is assigned read-only access to all of the pulse protocol waveforms on the system. The administrator may then select individual protocols and provide more access by giving both read and write privileges, or they may restrict access to a protocol altogether by giving the user no access at all. When a user is not given any protocol access they will not be able to see or select it from the Protocol screen when they login under their own user name. When protocols are added through the Protocol screen after a standard user account has already been created, all user accounts in the system will receive read-only access to the new protocol.

The administrator also has the ability to change a user's password. This is ideal for situations in which the user forgets his/her password and thus needs the password to be reset in order to login again (See Figure 11).

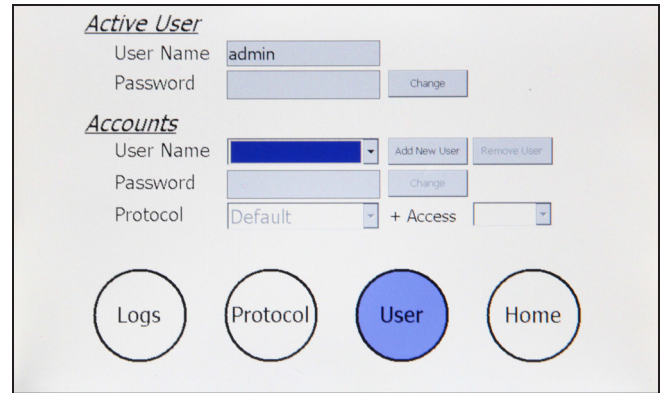


Figure 11: User Account Screen



**IMPORTANT!** The changes made on this screen will only be permanently saved when the HOME button is pressed to go back to the Welcome Screen. If modifications are made and system power is turned off while still viewing the screen, all changes will be lost.

# AgilePulse™ MAX System Operation

## Protocol Setup

Pulse protocols can be viewed and/or modified on the Protocol Screen which is accessed from the Welcome Screen:

### Touch SETUP

### Touch PROTOCOL

Parameters for up to three pulse groups are set by selecting the appropriate box and scrolling through the available values. Subsequent groups are activated by selecting more than one pulse in the number field and are conversely deactivated by selecting zero pulses in the number field. The adjusted protocol is saved to internal memory when the HOME button is touched (See Figure 12).

Only the system administrator has access to the “Add New” and “Remove” buttons which create new protocols and delete existing protocols respectively. When a new protocol is created, all user accounts in the system receive read-only access to it. The administrator can customize user access privileges for each protocol by navigating to the User Account Maintenance Screen, (see page 10).

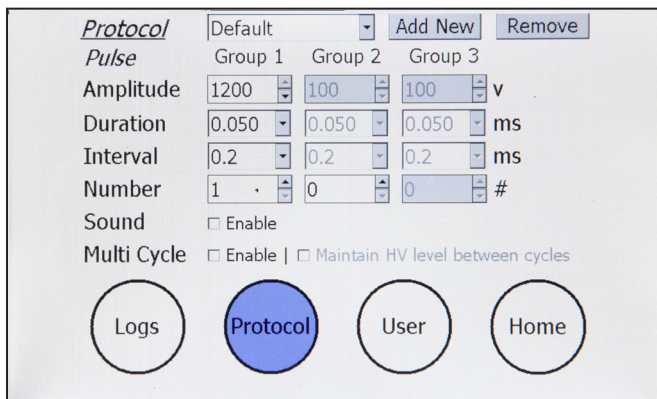


Figure 12: Protocol Screen



Caution

**IMPORTANT!** The changes made on this screen will only be permanently saved when the HOME button is pressed to go back to the Welcome Screen. If modifications are made and system power is turned off while still viewing the screen, all changes will be lost.

There are limitations on the range of parameters and interaction between various parameters.

The following rules must be observed:

### Pulse Amplitude

100 to 1200 Volts	10 Volt Steps	Pulse Width Range	50 μs to 1000 μs
100 to 300 Volts	10 Volt Steps	Pulse Width Range	1 ms to 10 ms

### Pulse Drop

Less than 2% of power supply voltage at 100 ohms

Less than 8% of power supply voltage at 10 ohms

### Pulse Widths in ms

0.05, 0.10, 0.30, 0.40, 0.50, 1, 2, 3, 4, 5, 10

### Pulse Width Droop

<7.5%

### Pulse Intervals in ms

0.2, 0.3, 0.4, 0.5

1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 1000

### Inter-group

Pulse interval must be ≥2X pulse width with a 0.2 ms minimum

### Intra-group

Pulse interval must be ≥2X pulse width with a 0.2 ms minimum

Time between end of pulse N and beginning of pulse N+1 must be  $> -0.04 \cdot \ln(V_{n+1}/V_n)$

### Number of Pulses

1 to 10 in steps of 1

### Number of Groups

1, 2 or 3

# AgilePulse™ MAX System Operation

## Log File Maintenance

The Log file maintenance screen is accessed from the Welcome screen:

### Touch SETUP

### Touch LOGS

All previous transfection run logs can be retrieved and saved onto a USB Key or deleted from internal memory. The interface consists of a series of chronological sub-folders from which the user is allowed to double-tap on the touch screen to descend through the hierarchy. The standard naming sequence is Year, Month, Day, followed by special folders with the time of day. These time folder icons show a “hand” underneath, meaning they contain all files generated from a single run. The “ ” folders allow the user to return to the previous level of hierarchy.

Special time folders display basic information on the right hand side of the screen when selected. Identification numbers, time of day, and status completion indicators help the user find the correct transfection run to download.

When selected by a single tap on the touch screen icon, any level of hierarchy can be saved to a USB Key inserted in the front panel by pressing the SAVE button. The hierarchy structure will be maintained and copied to the USB Key. The DELETE button is also available to remove the hierarchy from internal memory. This deletion is permanent and the lost files cannot be recovered.

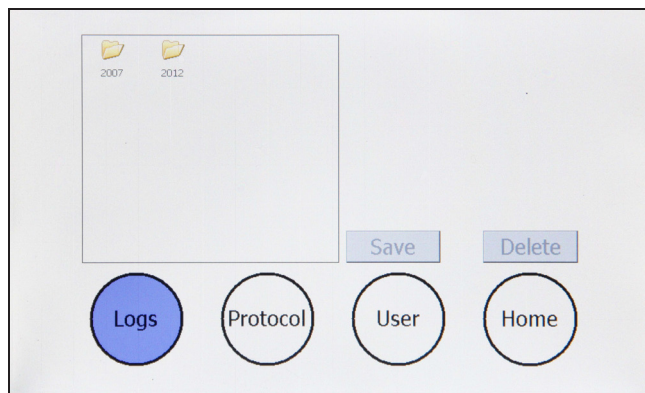


Figure 13: Log File Maintenance Screen



Caution

**The Saving and Deleting processes are slow and dependent on the number of files being saved or deleted. Do not use USB keys that will attempt to upload software onto the computer drive. Keys with <1 GB memory are recommended.**

# AgilePulse™ MAX System Operation

## Electroporation Protocol Delivery Logs

All collected data from the delivery is automatically stored on the internal Flash Memory and an inserted USB key under a unique chronological directory structure. Included items are an XML file containing raw detailed system and runtime data, a text log with basic runtime data, and a comma separated CSV file containing pulse monitor data.

The directory structure is constructed in a “AgilePulse MAX\Log\<Year>\<Month>\<Day>\<Time>” hierarchy format. A maximum of 20,000 delivery logs can be stored on the internal system memory.

	<b>Raw Data</b> (DV <Date> <Time>.xml)	<b>Text Log</b> (DV <Date> <Time>.log)	<b>CSV Data</b> (DV <Date> <Time>.csv)
Start and Stop Date	•	•	
Start and Stop Time	•	•	
Field 1	•	•	•
Field 2	•	•	•
Field 3	•	•	•
Process Completion Type	•	•	
Set Pulse Parameters	•	•	•
Average monitor pulse voltage		•	
Average monitor pulse current		•	
Average monitor pulse resistance		•	
All monitor pulse voltage samples	•		•
All monitor pulse current samples	•		•
Monitor width of each pulse	•	•	•
Monitor interval of each pulse	•	•	•
Check Reading	•	•	
System Serial Numbers	•		
System Software Versions	•		

# AgilePulse™ MAX System Operation

## Text Log

The text log contains the same information that appears on the delivery process screen after completion. Text log data is summarized in an easy to read format and is designed to provide quick feedback regarding the delivery completion. An example Log printout is presented in Figure 14.

Field	Description
Pulse	Pulse number
VSet	Voltage programmed to high voltage power supply
VMon	Voltage monitored during pulse
IMon	Current monitored during pulse
RMon	Equivalent load resistance calculated from voltage and current monitors $R = V / I$
DurSet	Pulse duration programmed to waveform generator
DurMon	Pulse duration monitored by independent time-auditor system
IntSet	Pulse interval programmed to waveform generator
IntMon	Pulse interval monitored by independent time-auditor system

Date Start: 2/12/2007 2:16:22 PM (2/12/2007 10:16:22 PM GMT)								
System Serial Number: 07-0120CS								
Cell Line: CHO								
Transfectant: Plasmid A								
Chamber: MD081101-1								
Process Completion Return Code: 0 (Success)								
Pulse	VSet	VMon	IMon	RMon	DurSet	DurMon	IntSet	IntMon
1	450	449	2.316	194	0.050	0.050	125.000	125.075
2	450	449	2.316	194	0.050	0.050	125.000	125.083
3	110	109	0.751	145	10.000	10.013	125.000	125.081
4	110	108	0.732	147	10.000	10.013	125.000	125.082
5	110	108	0.743	146	10.000	10.013	125.000	125.081
6	110	108	0.747	145	10.000	10.013	125.000	125.081
7	110	108	0.736	147	10.000	10.013	125.000	125.082
8	110	109	0.736	147	10.000	10.013	125.000	125.080
9	110	109	0.732	148	10.000	10.013	125.000	125.083
10	110	109	0.758	143	10.000	10.012	125.000	52.879
Load Reading: 204								
Date Stop: 2/12/2007 2:16:40 PM (2/12/2007 10:16:40 PM GMT)								
Time Elapsed: 00:00:18								

Figure 14: Text Log Screen



# AgilePulse™ MAX System Operation

## CSV Data

Detailed pulse information is exported from raw data and formatted to a standard comma separated file, primarily meant to be imported into Microsoft Excel® for further user manipulation. As an example, in Figure 15, a chart was made to displays the monitored amplitude samples of three 100 microsecond pulses run at 5 kHz. In this case, each pulse contains eight amplitude samples.

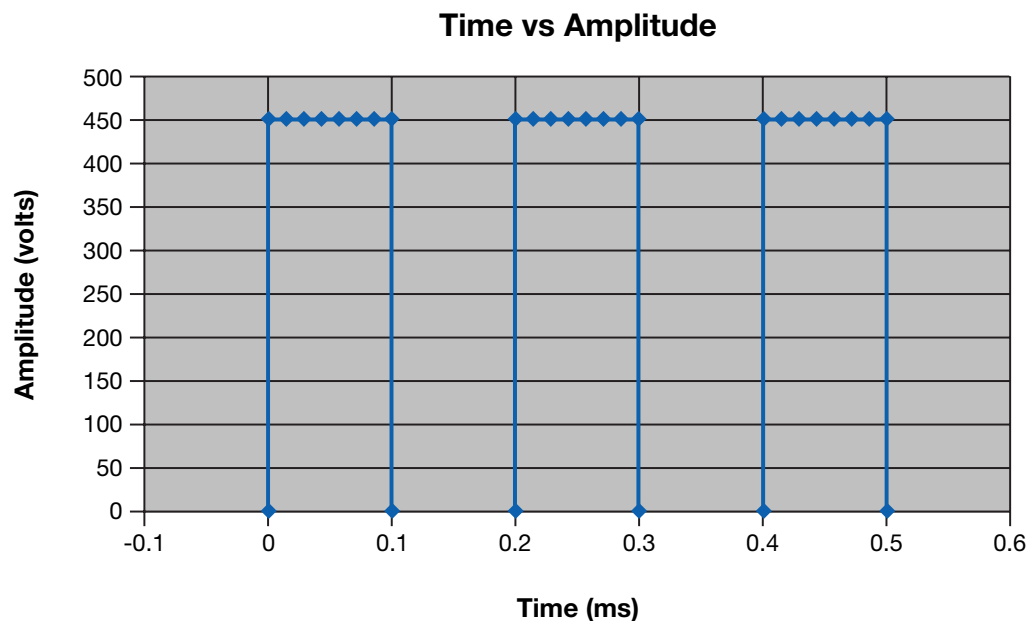


Figure 15: Sample Amplitude Analysis

# AgilePulse™ MAX System Operation

## Delivery Completion Return Codes

Several return codes are available following system shutdown describing how it took place.

Return Code	Description
0	Successful completion (Normal Operation)
1	Emergency Flash memory storage failure
2	Waveform protocol data integrity verification error
3	Waveform protocol mode not recognized
4	Protocol voltage below minimum specification
5	Protocol voltage above maximum specification
6	Protocol pulse width below minimum specification
7	Protocol pulse width above maximum specification
8	Protocol pulse interval below minimum specification
9	Protocol pulse interval above maximum specification
10	Protocol pulse number below minimum specification
11	Protocol pulse number above maximum specification
12	Protocol has a zero pulse group in between two non-zero pulse groups
13	Protocol pulse settings exceed maximum duty cycle
14	Low voltage power supply failure (Hard system turn-off)
15	High volt power supply voltage above maximum voltage
16	High volt power supply maximum current draw
17	Pulse voltage monitor detected off-zero voltage while no pulse was active
18	Pulse voltage monitor below maximum droop specification
19	Pulse voltage monitor above maximum overshoot specification
20	Pulse current monitor below minimum specification
21	Pulse current monitor above maximum specification
22	Independent time-audit system failed to respond to query
23	Independent time-audit system detected less pulses than expected
24	Independent time-audit system detected more pulses than expected
25	Independent time-audit system exceeded maximum measurable pulse number
26	Independent time-audit system detected excessive pulse duration variance
27	Independent time-audit system detected excessive pulse interval variance
28	Independent time-audit system data integrity verification failure
29	Reset button on front panel triggered
30	Control system watchdog timeout
251	High voltage timeout
252	Load estimation timeout
253	Delivery process was terminated prematurely
254	Control system experienced a system reset of unknown source
255	Load estimate outside of allowable range

# AgilePulse™ MAX System Operation

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## Transfection Process

The transfection process consists of the following steps:

1. **Preparing the polynucleotide**
2. **Preparing the cell suspension**
3. **Filling the chamber**
4. **Electroporation**
5. **Handling after electroporation**

## Preparing the Polynucleotide

Transfection efficiency is often directly related to polynucleotide concentration. However, the optimum concentration may vary depending on the material to be transfected. Some optimization will be required for each combination of polynucleotide and cell type. It is recommended that the polynucleotide be prepared in nuclease-free water rather than TE or other ionic buffers in order to maintain the low conductivity environment necessary for large volume transfection. The following are typical concentrations of polynucleotides that are used for electroporation:

- DNA 10 to 100 µg/mL
- mRNA 10 to 100 µg/mL
- siRNA 100 nM to 1 µM
- Oligonucleotides 100 nM to 20 µM

## Preparing Cell Suspensions for Large Volume Transfection

The following is a standard procedure for preparing cells for large volume electroporation. Cell washes may be carried out at 4°C. However, it is recommended that electroporation in Cytoporation® media be carried out at room temperature.

1. **Harvest cells from several T150 flasks, roller bottles or fermenter.**
2. **Centrifuge cells 300 X g for 10 minutes.**
3. **Pour off supernatant, resuspend cells and add 10 mL Cytoporation® Medium T.**
4. **Centrifuge cells 300 X g for 10 minutes.**
5. **Pour off supernatant, resuspend cells and add 10 mL Cytoporation® Medium T.**
6. **Centrifuge cells 300 X g for 10 minutes.**
7. **Resuspend cells at 10 to 100 million cells/ml in Cytoporation Medium® T or T4.**
8. **Add polynucleotide (DNA or RNA) to desired concentration.**

Efficient transfection can be achieved with cell densities ranging from 10 million cells/ml to 100 million cells/ml. It is important to remember that at high cell densities, leakage of ions from the cells during electroporation can contribute to significant increases in conductivity during pulse delivery.

## Filling the Chamber

The Large Volume Electroporation Chamber can be filled through one of the luer lock ports. It is recommended that a sterile syringe be used for filling and emptying of the chamber.

The Large Volume Electroporation Chambers may be re-used if properly cleaned after each use. Chambers can be sanitized by soaking in 1M NaOH (4% solution) for 10 minutes, then rinsing liberally with sterile water. Chambers are not autoclave-safe. Alternative cleaning procedures must be validated prior to use.

# AgilePulse™ MAX System Operation

## Electroporation

Place the Chamber in the Chamber Holder and apply the selected waveform protocol. It is recommended that electroporation protocols be optimized on a small-scale prior to transfection in large volumes. However, additional optimization is often required during the scale-up process. The following tables are provided as a starting point for protocol optimization. These protocols were established by BTX Harvard Apparatus or its collaborators by optimization in cuvettes. Additional information concerning transfection of specific cell types may be found in protocols 962 and 1012 on the BTX Harvard Apparatus web site: [www.btxonline.com](http://www.btxonline.com).

Pulse Parameters for Primary T Cells			
Cell line		Primary T cells	
Polynucleotide and concentration		mRNA, 168 µg/ml	
Pulse Parameters	Pulse Group 1	Pulse Group 2	Pulse Group 3
Amplitude V/cm	3000 V/cm	3000 V/cm	325 V/cm
Pulse Length	0.1 ms	0.1 ms	0.2 ms
Number of Pulses	1	1	4
Interval Time	0.2 ms	300 to 500 ms	2 ms

Pulse Parameters for Other Cell Lines								
			Pulse Group 1			Pulse Group 2		
Cell Line	Polynuc.	Conc. (µg/ml)	Amplitude (V/cm)	Duration (ms)	Pulse No.	Amplitude (V/cm)	Duration (ms)	Pulse No.
CHO-K1	Plasmid	50	2000	0.4	2	1000	0.8	2
HEK293	Plasmid	50	1600	0.2	2	800	0.8	4
3T3-L1	Plasmid	50	2000	0.4	2	1000	0.8	2
DBS-FRhl-2	mRNA	0.24	4500	0.1	2	1250	0.4	4
Neuro-2a	Plasmid	50	1260	0.4	2	630	1	2
K-562	Plasmid	25	2500	0.1	6			
VERO	Plasmid	50	2000	0.2	2	1000	1	4

## Cell Handling After Electroporation

Cytoporation® Media are non-toxic to cells but do not have nutrients to support cell growth. Therefore, cells should be kept in Cytoporation® Medium for a limited time. It is recommended that cells be suspended in Cytoporation® Media for a maximum of one hour prior to electroporation.

It is recommended that cells be returned to culture medium soon after electroporation. However, transfection efficiency is often enhanced by allowing the cells to rest for 1 to 2 minutes before adding culture medium. The cells can be either washed in culture medium to completely remove the Cytoporation® Medium or the cells can be diluted in culture medium without removing the Cytoporation® Medium. It is recommended that at least five parts culture medium be added for every one part Cytoporation® Medium.

# AgilePulse™ MAX Tutorial

## Applications

The AgilePulse™ MAX system is used to deliver material into cells using pulsed electric fields. It is a large capacity system capable of treating up to 10 ml of cell suspension. The AgilePulse™ MAX system was specifically designed for the transfection of cells with polynucleotides. However, a variety of charged material can potentially be delivered into cells.

## DNA/RNA Delivery into Cells Using Electroporation

### General Electroporation Discussion

Electroporation uses pulsed electric fields to create microscope pathways (pores) in bio-membranes. Their presence allows macromolecules, ions, and water to pass from one side of the membrane to the other. As the right side bar shows, 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, pores form that permit material outside of the cell to enter. If the electric field is promptly removed, the pores close and the membrane reseals. If the electric field duration is too long, the pores increase in size and number and the cell is killed.

Electroporation was first used to introduce small molecules into cells in the early 1980s and quickly became a popular research method. By the late 1980s electroporation applications were being developed for multi-cellular tissues. In the early 1990s Lluís Mir of the Institute Gustave-Roussy was the first to use electroporation in a human trial to treat external tumors.

Efficient electroporation depends on proper selection of electric field waveforms. Pores begin to form in about a microsecond and reseal in seconds to minutes. Therefore, duration of the applied electric fields are typically in the range of tens of microseconds to tens of milliseconds.

Induction of electropores is affected by a number of factors. Pores are induced when the product of the pulse amplitude and the pulse duration is above a lower limit threshold. Normal cell-to-cell biological variability can cause some cells to be more sensitive to electroporation than other cells. Although other factors are involved, the lower limit threshold for a particular cell type is largely dependent on the reciprocal of cell size (See Figure 16).

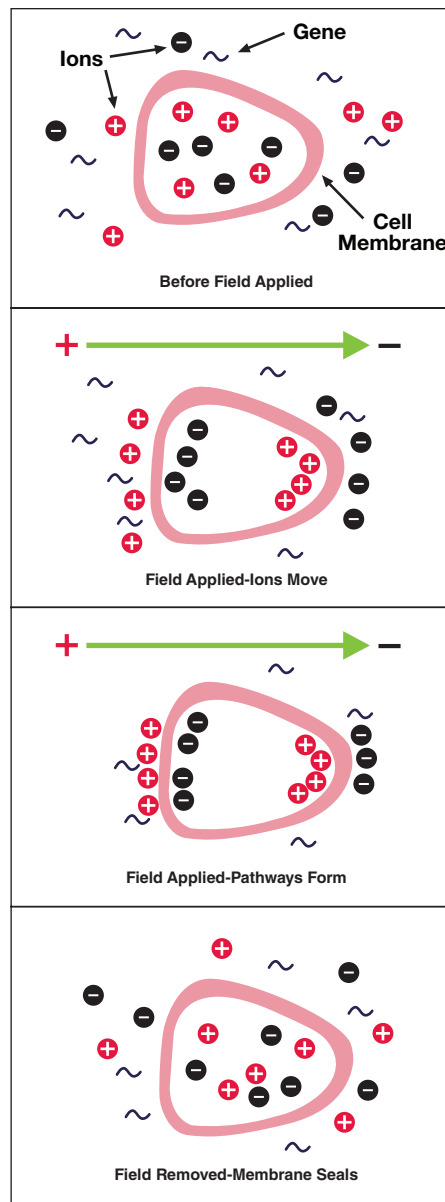


Figure 16: Electroporation

# AgilePulse™ MAX Tutorial

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:

$$TMV = -\frac{3}{2} E r | \cos \alpha |$$

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 μm radius, the required electric field intensity is:

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

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

Another important point to consider is the generation of heat during electroporation. Heat production is directly related to current intensity which is, in turn, dependent on the conductivity of the material through which the electric field is applied. Standard saline solutions such as PBS and many tissue culture media are highly conductive and, thus, will generate considerable amounts of heat when used in cell electroporation. Excessive heating can be detrimental to cell viability. The effects of heating can be reduced by using a low conductivity medium such as BTX's Cytoporation® medium to resuspend cells prior to electroporation (see also Importance of Medium Conductivity in Electroporation on page 7 for other important issues related to conductivity).

Although electroporation is an effective method for introducing macromolecules into 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).

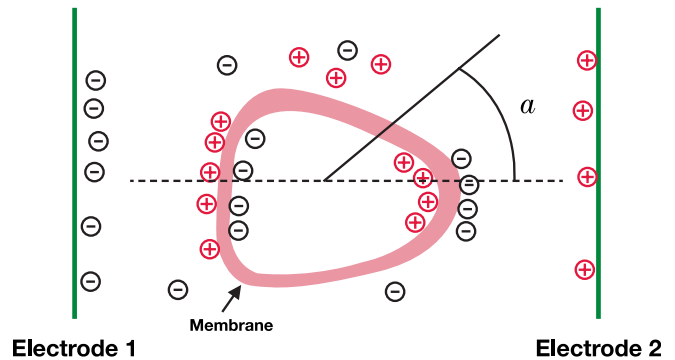


Figure 17: Transmembrane Voltage

## PulseAgile™ Electroporation

PulseAgile™ electroporation differs from standard electroporation in that it allows control of pulse parameters from pulse to pulse within a single delivery sequence. This means that values for pulse voltage, pulse duration, interval between pulses, and number of identical pulses in a subset can be programmed to change during a series of pulses. In practice, PulseAgile™ is usually used to deliver high electric field pulses of short duration followed by lower electric field pulses of longer duration. This method increases electroporation efficiency in numerous applications and is covered by U.S. patents 6,010,613 and 6,078,490.

Physical principles of importance to electroporation are:

1. **Applied pulsed electric fields of optimal magnitude and duration induce a transient state of permeability in cells immersed in that electric field (Neumann, 1972).**
2. **An applied electric field can move charged particles in an ionic medium by electrophoresis (Moyer, 1936).**
3. **An electrical current is required to maintain an electric field in an ionic medium (Ohm's Law).**
4. **Heat production by an electrical current in an ionic medium is proportional to the square of the current (Joule's First Law).**

These principles can be used to optimize electroporation. All four of these physical processes are active during the entire time that electric fields are applied to the area around cells. However, they vary in importance at different times during electroporation.

## Induction of a State of Permeability

Applying an electric field across a cell causes a redistribution of internal ions because cell membranes act as insulators. Ions move according to their charge within the electric field, but remain trapped within the cell thus accumulating at the poles in line with the electric field. This polar accumulation of ions within the cell creates an electrical potential across the cell membrane. When the transmembrane voltage exceeds a threshold of approximately one volt, conformational changes within the membrane induce a state of permeability.

The potential across a cell membrane is not equal along the entire surface of the cell. It is highest when the surface of the cell membrane is perpendicular to the electric field (in line with the electric field) and lowest when the surface of the cell membrane is parallel or tangential to the electric field (out of line with the electric field). The variation of transmembrane potential in an electric field as described above is related to the cosine of  $\alpha$  with the greatest transmembrane potential at the poles of the cells in line with the electric field and the least transmembrane potential 90 degrees from the poles (See Figure 17).

Increasing the electric field from one value above threshold to a higher value above threshold will induce permeability in a larger surface area of the cell. This is because of the dependence upon the cosine shown in the formula above. Thus, high electric fields are used in first pulses to permeabilize a large area of the cell membrane.

The permeabilized area on each end of a cell will be asymmetrical. It will be slightly larger on the anode (+) side than the cathode (-) side. This is because the cell has a slight natural negative charge across its membrane (Golzio, 2002).

## Movement of DNA or RNA Across Cell Membranes

The principle force influencing movement of polynucleotides across a cell membrane during electroporation is electrophoresis (Klenchin, 1991). This has been shown both in vitro (Andreason, 1989) and in vivo (Satkuskas, 2002). Electrophoresis is the movement of charged particles in a solution in response to an electric field. Movement of particles by electrophoresis is proportional to the total applied charge, which is in turn proportional to the magnitude of the electric field times the duration of the electric field. Assuming other factors such as solution viscosity are constant, the movement is linearly related to the total charge. This means that total distance traveled is approximately the same when half of a given electric field is applied for twice a given time.

## Minimizing Heat Production with Waveform

Joule's first law states that ohmic or Joule heating is proportional to electrical resistance times the square of the current. Joule heating is a common cause of cell death during electroporation, so excessive increases in temperature due to this effect should be avoided. Following enough poration of the cell membrane to physically allow transfectant entry, a subsequent reduction in electric field will continue to move the transfectant by way of electrophoresis while generating much less heat. For example, a half decrease in electric field will reduce heat generated four fold.

## Putting the AgilePulse™ Concepts Together

A summary of the proportional relationships of physical factors during electroporation are:

- 1. The area of a cell permeabilized by electroporation is positively correlated to the magnitude of an applied electric field (less than linearly because of the cosine in the formula).**
- 2. The movement of a charged molecule (such as DNA) in a solution in an electric field is linearly related to the total applied charge.**
- 3. Heat produced during electroporation is proportional to the square of the applied current (or of the applied voltage if the electrical resistance is constant).**

Given these relationships, maximum cell permeability and maximum DNA delivery can be achieved by delivering a series of brief high electric field pulses at the beginning of electroporation to induce permeability in a large area of the cells followed by delivery of a series of lower electric field pulses to induce maximum movement of DNA with minimal heating.

## Importance of Medium Conductivity in Electroporation

When using standard cuvettes in electroporation, medium conductivity is generally not a critical issue. However, for large volumes medium conductivity quickly becomes critical. As the volume of cell suspension gets larger the resistance of the bulk solution gets smaller. This relationship can be represented mathematically as follows:

$$R = \rho * \frac{gap}{area} \text{ ohms}$$

where:

- R* is the resistance in ohms of the solution in the chamber.
- gap* is the spacing between two parallel electrodes.
- area* is the active area of the electrodes that are in contact with the cell suspension (the areas of the two electrodes are identical).
- $\rho$  is the resistivity of cell suspension in the chamber with units ohm-cm.

Engineers and physicists generally use resistivity, biologists often use conductivity:

$$Conductivity = \sigma \frac{1}{resistivity} \text{ Siemens/cm (often converted to milliSiemens / cm)}$$

Since it is often difficult to make a direct measurement of the active area of the electrodes in a cuvette or electroporation chamber, a more practical equation is needed. The volume of cell suspension added to a cuvette can be represented as follows:

$$R = 1/\sigma \frac{gap^2}{volume} \text{ ohms}$$

where:

- $\sigma$  is in S/cm (if in mS/cm then s/103, if in  $\mu$ S/cm then s/106).
- gap* is in cm.
- volume* is in ml.



# AgilePulse™ MAX Tutorial

The table below provides some insight into how conductivity and volume affect the resistance in an electroporation chamber. Examples are given for a standard 4 mm cuvette with a maximum volume of 800 µl, a 6 mm gap sterile chamber with a maximum volume of 6 ml, or a 4 mm flatpack with a maximum volume of 10 ml. The conductivities of Opti-MEM I and RPMI-1640 are similar to the conductivity of PBS.

Medium*	Conductivity, S (mS/cm)	Gap (cm)	Volume (mL)	Resistance, R (ohms)	Pulse Current @ 1000 V (Amps)
T	0.08	0.4	0.8	2500	0.5
T-4	3.4	0.4	0.8	59	17
Opti-MEM I	N/D	0.4	0.8	22	45
RPMI 1640	N/D	0.4	0.8	15.8	63
PBS	16.6	0.4	0.8	12	83
T	0.08	0.4	5	400	3
T-4	3.4	0.4	5	9.4	106
PBS	16.6	0.4	5	1.9	519
T	0.08	0.6	2	2250	0.4
T-4	3.4	0.6	2	52.9	19
PBS	16.6	0.6	2	11	92
T	0.08	0.6	6	750	1.3
T-4	3.4	0.6	6	17.6	57
PBS	16.6	0.6	6	4	277

For practical reasons, as the bulk of volume to be electroporated increases, the conductivity must be decreased. Otherwise, current requirements will rapidly exceed available capacity.

# AgilePulse™ MAX Tutorial

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## Cytoporation® Medium Properties

### Conductivity

The conductivity of most common electroporation buffers range from 10 to 20 mS/cm; these conductivities are too high for electroporation of large volumes. Cytoporation® media were developed to address this problem. Although very low conductivity is desired for large volumes, experimental data indicate that some cell types require a moderately conductive environment to achieve optimal transfection efficiency. The trade-off between maximum volume and optimal efficiency must be considered when choosing a medium for large volume electroporation.

### Media Formulas

There are two Cytoporation® formulas currently available: Cytoporation® Medium T at 0.08 mS/cm and Cytoporation® Medium T-4 at 3.45 mS/cm. These media are produced under strict standards and are listed with the FDA under Master File Number MAF-1508.

Some cell types can be sensitive to the conductivity of the electroporation buffer. Therefore, optimization is usually required to determine the most effective formulation for a given cell type.

Another point to consider is that ions within the cells will leak out when the cell membranes become porated. This will contribute to an increase in conductivity of the extracellular solution during the course of electroporation. This effect is especially important to consider when working with high concentrations of cells (i.e. 100 million cells/ml).

## Other Important Cytoporation® Medium Properties

In addition to low conductivity, Cytoporation® Media have other properties that contribute to successful electroporation such as physiological pH and normal osmolarity. The pH is moderately buffered, but addition of any reagents that could affect pH should be avoided. The osmolarity of Cytoporation® Media is compatible for animal cells. Addition of small molecule reagents (ionic or non-ionic), if added in substantial amounts to the medium, may adversely affect osmolarity.

Cytoporation® Media contain proprietary ingredients designed to improve the viability of cells during electroporation.

Cytoporation® Media are RNase and DNase free. Every batch is tested and certified free of the presence of both enzymes.

Cytoporation® Media are low endotoxin media. Endotoxin will adversely affect transfection of cells using electroporation. Therefore a low endotoxin medium is essential.

*The AgilePulse™ MAX system was designed to be used with the Cytoporation® Media offered by BTX Harvard Apparatus. The use of other buffers or media may invalidate the warranty.*

# Specifications

<b>AgilePulse™ MAX</b>	
<b>Current Ratings</b>	
Input Voltage frequency Ratings	100 to 240 VAC, 50/60 Hz
Power Ratings	5A
Dimensions (W x H x L)	30.5 cm x 15.9 cm x 36.8 cm (12 in x 6.25 in x 14.5 in)
Weight	5.7 kg (12.5 lb)
Warranty	2 years
Operating Temperature	4°C to 40°C (40°F to 104°F)
Storage Temperature	-10°C to 70°C (14°F to 158°F)
Operating Humidity	20% to 80% RH, non-condensing
Storage Humidity	20% to 80% RH, non-condensing
Mode of Operation	Continuous
Classification	Class I
Pollution	IP2X
Installation	Category II
Screen Technology	Capacitive touch
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 and RoHS

*\*All data automatically stored in internal memory and may be downloaded to an external USB Key.  
Maximum Data Logs stored and retrievable from internal flash memory >20,000.*

# Maintenance

The AgilePulse™ MAX system requires no special maintenance other than keeping it clean.

If the surface of the touch screen display or enclosure needs to be cleaned, use a standard (non-ammonia) glass cleaner or mild detergent with warm water and a soft, lint free paper or cloth towel. Do not apply the cleaning solution directly to the screen or enclosure, to avoid liquid running into other parts of the cabinet. Put a small amount of cleaner on the towel and gently rub the screen or enclosure. Avoid hard rubbing, abrasives, or harsh solvents like alcohol or ammonia.

## Fuse Replacement

1. Make sure the power cord is disconnected from the main supply before servicing the fuse.
2. Replace fuse with (240V/5A slo-blo) 5 x 20 mm.

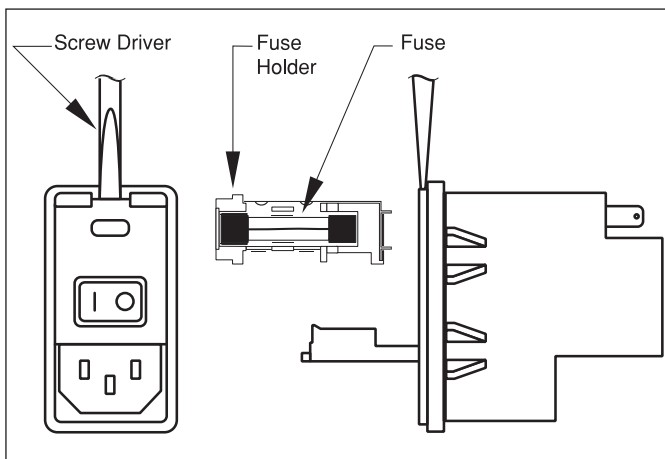


Figure 18: Fuse Replacement

## Electrode Care and Cleaning

Needle Array electrodes are disposable arrays made of medical grade plastic with surgical steel needles. However needle arrays can be used up to 500 pulses if cleaned immediately following use.

### 7.2.1 Post Electroporation Electrode Care

- Rinse electrode contact area in warm water.
- Wash with dilute detergent (1% V/V). Needles may be brushed gently with a soft brush (for example toothbrush).
- Rinse well with lab grade deionized water.
- Air dry.
- Check for bent or depressed needles; all needles should be equal length.
- Discard any needle arrays with bent or uneven length needles.

### 7.2.2 Electrode Sterilization

- Soak needle end of electrode for 10 minutes in 4% Sodium Hydroxide (suggested use vessel such as a petri dish).
- Rinse well with sterile lab grade water.
- Spray with 70% alcohol.
- Air dry.
- Replace needle protection cap for storage until next use.

# Ordering Information

Item No.	Description	Qty.
<b>Electroporation Systems</b>		
<b>47-0200N</b>	AgilePulse MAX Large Volume Transfection System, Complete*	1 Ea
<b>47-0201N</b>	AgilePulse MAX Waveform Generator, Generator Only	1 Ea

\*Includes large volume chamber stand, safety stand, 5 ml chamber, Cytoporation medium T (2X), 500 ml and 4 mm gap cuvettes

Item No.	Description	Qty.
<b>Accessories</b>		
<b>47-0002</b>	BTXpress Cytoporation Low Conductivity Medium T, 500 ml volume	
<b>47-0202N</b>	AgilePulse MAX Stand for Large Volume (5 ml) Chambers	
<b>47-0203</b>	AgilePulse MAX Safety Stand for Cuvettes	
<b>47-0204N</b>	AgilePulse Large Volume Chamber, 6 mm gap, 5 ml, 2 ports with fittings	
<b>47-0206</b>	Flatpack Chambers, 4 mm gap, 10 ml volume, pkg. of 10	
<b>47-0010</b>	AgilePulse Large Volume Chamber, 6 mm gap, 5 ml, 4 ports	
<b>Service Offerings</b>		
<b>45-9996</b>	AgilePulse 1 Year Extended Warranty	1 Ea
<b>45-0909</b>	AgilePulse System Validation Certificate	1 Ea

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