

AgilePulse™ MAX

Large Volume
Transfection System

USER'S MANUAL



BTX®

a division of Harvard Bioscience, Inc.

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AgilePulse™ MAX Large Volume Transfection System

RESEARCH ONLY

BTX Harvard Apparatus

84 October Hill Rd
Holliston, MA 01746, USA

phone 1-508-893-8999 • fax 1-800-429-5732 • www.btxonline.com

BTX Warranty

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

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

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

Warranty is void if the AgilePulse™ MAX instrument is changed in any way from its original factory design or if repairs are attempted without written authorization by BTX - HARVARD APPARATUS.

Warranty is void if parts, connections or electrodes not manufactured by BTX - HARVARD APPARATUS are used with the AgilePulse™ MAX instrument. If a defect arises within the warranty period, promptly contact BTX - Harvard Apparatus, 84 October Hill Road, Building 7, Holliston, Massachusetts, USA 01746-1388 using our toll free number 1-800-272-2775 (US Only) or 508-893-8999

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

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





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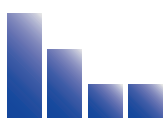
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Caution Notice

This instrument contains a high voltage power supply adjustable to 1,000 volts. 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 instrument is placed into operation.**
- **Removing the cover may 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 a problem occurs during a run, push the STOP/RESET 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**.



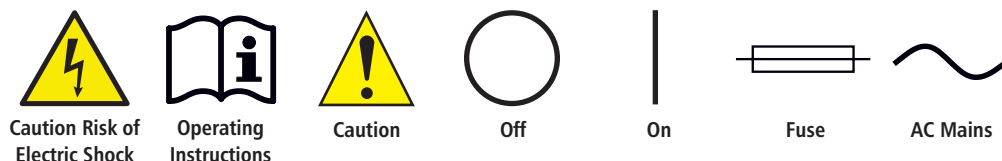
1 Introduction

The AgilePulse MAX™ system was developed to transfect large volumes of cells for commercial applications using pulsed electric fields (electroporation). The system has been designed for use in commercial processing.

The system has four components, the Waveform Generator Subsystem, the ex vivo large volume Chamber Electrode Subsystem, low conductivity Cytoporation® medium and 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.

Note: The AgilePulse MAX Waveform Generator Subsystem contains a high voltage power supply and was designed with safety features to protect the user and the equipment. If used properly, the AgilePulse MAX Waveform Generator is a safe and reliable instrument.

Front and Back Panel Symbols



The AgilePulse MAX™ system is intended for laboratory use only and can be used in research and development applications.

The system was designed to meet the standards for electromagnetic compatibility (EMC) and safety requirements 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.

The following material is contained in this User Manual:

CHAPTER 2 – Items of Particular Interest

CHAPTER 3 – AgilePulse MAX™ Tutorial

CHAPTER 4 – AgilePulse MAX™ System Components and Set-up

CHAPTER 5 – AgilePulse MAX™ System Operation

CHAPTER 6 – Customer Service



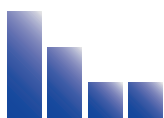
2 Items of Particular Interest

The AgilePulse MAX™ Large Volume Transfection System is the most sophisticated system available. The system is available from BTX Harvard Apparatus, Inc.

The following are important:

1. **The system shall only be used as described in this User Manual, the user is required to read the User Manual and undergo training before use.**
2. **Certain concepts and processes are covered by patents, patents pending, know-how and trade secrets**
3. **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.**
4. **Do not connect or disconnect the Chamber electrode from the waveform generator when the pulse waveforms are being applied.**
5. **Do not put your fingers or any other object on the Chamber while the waveforms are being applied.**
6. **This system produces voltages as high as 1200 volts and may cause injury or death if used improperly.**
7. **The system shall not be used in the presence of flammable gas.**
8. **The system is not for use on humans**

BTX Harvard Apparatus welcomes any comments from users to improve this manual.



3 AgilePulse™ MAX Tutorial

3.1 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 20 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.

3.2 DNA/RNA Delivery into Cells Using Electroporation

3.2.1 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 1980's and quickly became a popular research method. By the late 1980's electroporation applications were being developed for multi-cellular tissues. 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.

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.

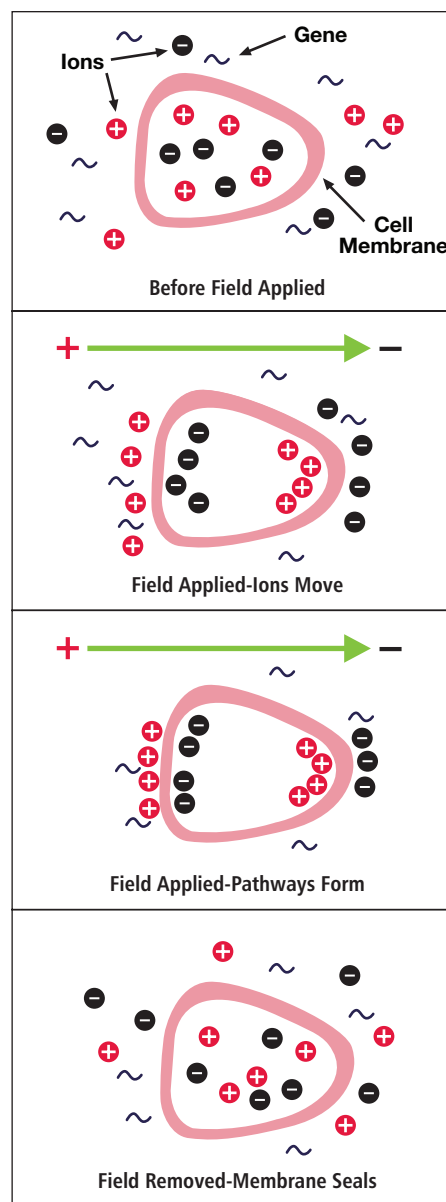


Figure 3-1: Electroporation



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3.2 DNA/RNA Delivery into Cells Using Electroporation (cont'd)

3.2.1 General Electroporation Discussion (cont'd)

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 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 (see also Section 3.4 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).

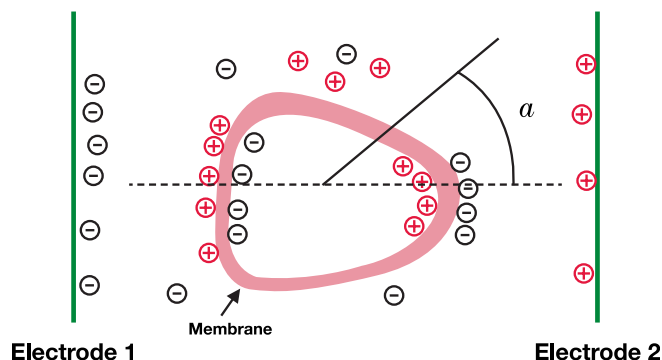


Figure 3-2: TRANSMEMBRANE VOLTAGE

3.3 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.



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3.3 PulseAgile® Electroporation (cont'd)

3.3.1 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 θ 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 3-2)

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).

3.3.2 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 (Satkauskas, 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.

3.3.3 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.

3.3.4 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.



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3.4 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:

$$R = r \frac{gap}{area} \text{ ohms}$$

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)
- r is the resistivity of cell suspension in the chamber with units ohm-cm

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

$$\text{Conductivity} = s \frac{1}{\text{resistivity}} \text{ Siemens/cm}$$

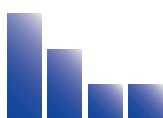
= (often converted to milliSiemens / cm)

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:

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

where:

- S is in S/cm (if in mS/cm then $s/10^3$, if in $\mu S/cm$ then $s/10^6$)
- gap is in cm
- $volume$ is in mL



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3.4 Importance of Medium Conductivity in Electroporation (cont'd)

The table below provides some insight into how conductivity and volume affect the resistance in an electroporation chamber. Examples are given for both a standard 4 mm cuvette with a maximum volume of 800 μ L and a 20 mL chamber that contains either 5 mL or 20 mL volumes.

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

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.



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3.5 Cytoporation® Medium Properties

3.5.1 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 was 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.

3.5.2 Media Formulas

There are a number of 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).

3.5.3 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.



3 AgilePulse™ MAX Tutorial

3.6 References

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4 AgilePulse™ MAX System Components & Set-Up

4.1 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**
- **6 ml large volume chambers (Cable, Base and Chamber Electrode)**
- **Safety Stand (Used for Cuvette Electroporation)**
- **Cytoporation® Medium**
- **User Manual**

4.2 The AgilePulse™ MAX Waveform Generator

The Waveform Generator is a computer with high voltage pulsing circuits (Figure 4-1). The computer runs mobile Windows® 6.0 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. All User inputs and outputs are via the touch screen.

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 4-1: Waveform Generator



4 AgilePulse™ MAX System Components & Set-Up

4.2 The AgilePulse MAX Waveform Generator (cont'd)

4.2.1 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 4-2).

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 4-2: Waveform Generator Front Panel



Caution

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

4.2.2 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.

4.2.3 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 for connecting a syringe to load cell suspension sample into chamber. This 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 electroporation. The cells, Cytoporation® medium and material to be transfected are mixed and placed in the chamber via the lower luer lock connector 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 4-3)

The chamber can be used with a minimum of 2 mL and a maximum of 6 mL. The chamber should be used one time unless a validated cleaning procedure is established, documented and allowed by the protocol.



Figure 4-3: Chamber and Base



4 AgilePulse™ MAX System Components & Set-Up

4.2 The AgilePulse™ MAX Waveform Generator (con'td)



Figure 4-4: Cytoporation® Medium

4.2.4 Cytoporation® Medium

BTX Harvard Apparatus provides a low conductivity media that are especially formulated for large volume electroporation. There are currently three formulas available with different conductivities:

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

See Chapter 3 for more details about conductivity and large volume transfection.

4.3 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.**

4.3.1 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. It may be necessary to check the fuse on the back panel of the Waveform Generator. If it is faulty, replace it with the exact type fuse (240V/5A, slo-blo) 5 x 20 mm. 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:

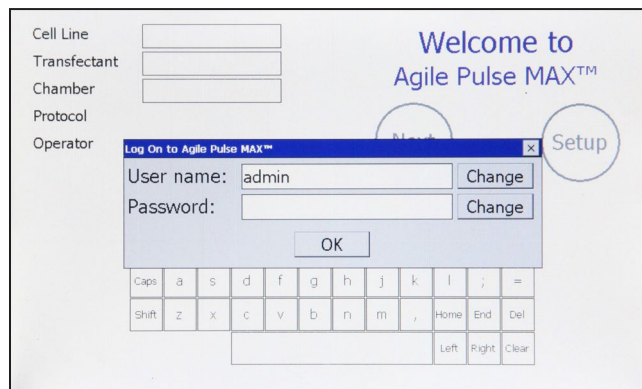


Figure 4-5: Start Up Screen Display



4 AgilePulse™ MAX System Components & Set-Up

4.3 Setting Up the System (cont'd)

4.3.2 Touch Screen Cleaning

If the surface of the touch screen display 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, to avoid liquid running into other parts of the cabinet. Put a small amount of cleaner on the towel and gently rub the screen. Avoid hard rubbing, abrasives, or harsh solvents like alcohol or ammonia.

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

www.btxonline.com



5 AgilePulse™ MAX System Operation

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

5.1 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**

5.1.1 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 5-1 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.

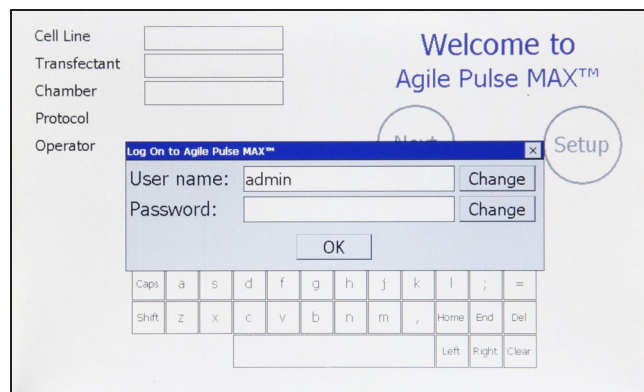


Figure 5-1: Login Screen

To enter User names and Passwords, touch the Change buttons to activate a virtual keyboard.

User Name: Enter the user’s name to login

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.



Caution

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.



5 AgilePulse™ MAX System Operation

5.1 Waveform Generator (cont'd)

5.1.2 Welcome Screen – Sample Identification Input

A Welcome Screen will be presented following successful user login 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.

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 Section 5.1.5). 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 5-2: Welcome Screen

5.1.3 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 6 mLs 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 5-3: 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.

Figure 5-3: Pulse Delivery Screen – Pre-Pulse

Figure 5-4: Pulse Delivery Screen – Post-Pulse



5 AgilePulse™ MAX System Operation

5.1 Waveform Generator (cont'd)

5.1.4 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.

Figure 5-5: User Account 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.



5 AgilePulse™ MAX System Operation

5.1 Waveform Generator (cont'd)

5.1.5 Protocol Setup

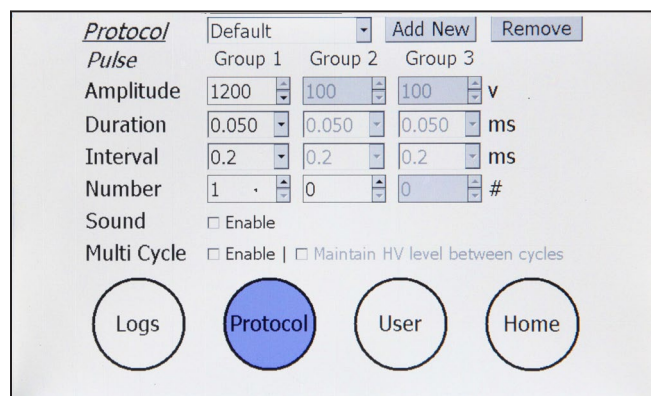
Pulse protocols can be viewed and/or modified on the Protocol Screen which is accessed from the Welcome Screen:

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.

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 Maintenance screen (see Section 5.1.4).



Protocol	Default	Add New	Remove
Pulse	Group 1	Group 2	Group 3
Amplitude	1200	100	100
Duration	0.050	0.050	0.050
Interval	0.2	0.2	0.2
Number	1	0	0
Sound	<input type="checkbox"/> Enable		
Multi Cycle	<input type="checkbox"/> Enable <input type="checkbox"/> Maintain HV level between cycles		

Buttons: Logs, Protocol, User, Home

Figure 5-6: 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 μ s to 1000 μ s
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 $\geq 2X$ pulse width with a 0.2 ms minimum

Intra-group

Pulse interval must be $\geq 2X$ pulse width with a 0.2 ms minimum

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

Number of Pulses

1 to 10 in steps of 1

Number of Groups

1, 2 or 3



5 AgilePulse™ MAX System Operation

5.1 Waveform Generator (cont'd)

5.1.6 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 5-7: Log File Maintenance Screen



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.



5 AgilePulse™ MAX System Operation

5.1 Waveform Generator (cont'd)

5.1.7 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.

	Raw Data (DV <Date> <Time>.xml)	Text Log (DV <Date> <Time>.log)	CSV Data (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	√		



5 AgilePulse™ MAX System Operation

5.1 Waveform Generator (cont'd)

5.1.7 Electroporation Protocol Delivery Logs (cont'd)

5.1.7.1 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 5-8.

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 5-8: Text Log Screen



5 AgilePulse™ MAX System Operation

5.1 Waveform Generator (cont'd)

5.1.7 Electroporation Protocol Delivery Logs (cont'd)

5.1.7.2 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 5-9, 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.

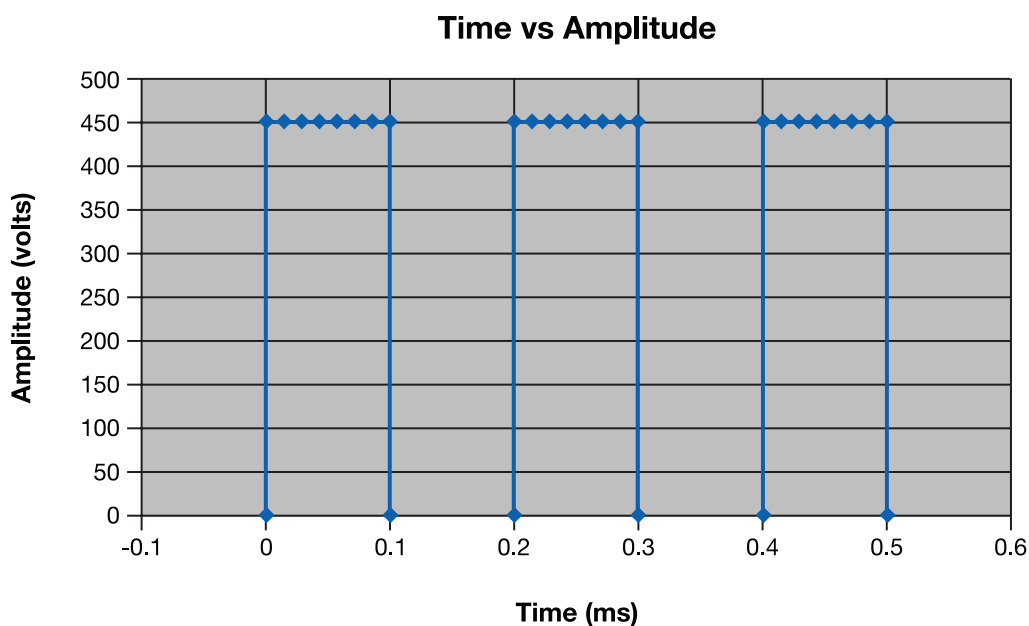


Figure 5-9: Sample Amplitude Analysis



5 AgilePulse™ MAX System Operation

5.1 Waveform Generator (cont'd)

5.1.8 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



5 AgilePulse™ MAX System Operation

5.2 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

5.2.1 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 $\mu\text{g/mL}$
- mRNA 10 to 100 $\mu\text{g/mL}$
- siRNA 100 nM to 1 μM
- Oligonucleotides 100 nM to 20 μM

5.2.2 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 20 mL Cytoporation[®] Medium T.
4. Centrifuge cells 300 X g for 10 minutes.
5. Pour off supernatant, resuspend cells and add 20 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 T-4
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.

5.2.3 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.



5 AgilePulse™ MAX System Operation

5.2 Transfection Process (cont'd)

5.2.4 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 table is 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 on the BTX Harvard Apparatus web site: www.btxonline.com.

Cell Line	Polynuc.	Conc. (µg/ml)	Pulse Group 1			Pulse Group 2		
			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

5.2.5 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 1-2 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.



6 Customer Service

6.1 Limited Warranty

The terms of the warranty are covered in the BTX warranty.

6.2 Customer Service

If the user believes that there is a defect in the BTX product, the customer should contact BTX Technical Support at **800-272-2775** or email **techsupport.btx@harvardapparatus.com**. If the system needs to be returned to BTX, please contact Technical Support for a Return Material Authorization (RMA) number.

6.3 Parts and Accesories

Order #	Description
47-2000	AgilePulse™ MAX System: Includes AgilePulse™ Max pulse generator, cuvette safety stand, electroporation stand, 2x 6 ml chambers with 6 mm gap, manual and CD with training videos
47-0201	AgilePulse™ Max Generator ONLY
47-0202	Electroporation Stand for 6 ml chambers
47-0203	Saftey stand for cuvettes
47-0010	6 ml chambers, 6 mm gap each
47-0002	Cytoporation Media T



Declaration of Conformity

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

We herewith declare that the following product:

Product Name: *BTX AgilePulse™ ID BTX AgilePulse™ IM BTX AgilePulse™ MAX*
Model No.: *Catalog # 47-04xx Catalog # 47-05xx Catalog # 47-02xx*

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

Application of
Council Directive(s): *2006/95/EC Low Voltage Directive
2004/108/EC Electromagnetic Compatibility Directive
2011/65/EU RoHS Compliant*

Standard(s) to which
conformity is declared:

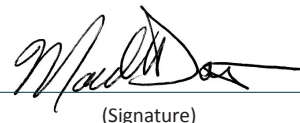
Safety: *IEC 61010-1:2010 (3rd Edition)*
Emissions/Immunity: *EN 61326-1:2013
FR47 FCC Part 15 Sub Part B (2011)
EN 61000-4-2:1995 + A1:1998+A2:2001
EN 61000-4-3:2002
EN 61000-4-4:2004
EN 61000-4-5:1995+A1:2001
EN 61000-4-6:2007
EN 61000-4-11:2004
EN 61000-3-2:2000
EN 61000-3-3:1995+A1:2001
CISPR11:2003+A1:2004, Group 1, Class A*

EMC and Safety compliance were evaluated by Intertek/ETL Semko

Reference test report
file numbers: *100587731 BOX-001, -003, -004*

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

Place: *United States of America*
Date: *April 14, 2015*



(Signature)

Mark Davis

(Full Name)

Director, Global MPE

(Position)

