

User's Manual

ECM 399 Electroporation System



45-0050

ECM 399 Electroporator only

45-0000

ECM 399 Electroporation System

BTX[®]

a division of Harvard Bioscience, Inc.

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Ярославль (4852)69-52-93

Россия +7(495)268-04-70

Казахстан +7(727)345-47-04

Беларусь +3(75)257-127-884

Узбекистан +998(71)205-18-59

Киргизия +996(312)96-26-47

эл. почта: bxt@nt-rt.ru || сайт: <https://btx.nt-rt.ru/>

General Information (Continued)

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 ECM 399 Electroporation System is intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) and safety.

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

General Safety Summary

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

To Prevent Hazard or Injury

USE PROPER POWER CORD

Use only the power cord specified for this product and certified for the country of use.

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.

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 EXPOSURE TO 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 manage the connection and disconnection of devices.

PLACE PRODUCT IN PROPER ENVIRONMENT

Refer to installation instructions for details on installing the product to ensure proper ventilation.

OBSERVE ALL WARNING LABELS ON PRODUCT

Read all labels on product to ensure proper usage.

Electrical & Technical Specifications

Standard Capabilities:

Display	Type: 16-character liquid crystal LCD backlit
Power Source	
Voltage	100 to 240 VAC, 50/60 Hz, CAT I
Power	800 W
Fusing	2.5 A, T rating 250 V

Environmental Characteristics:

Intended Use	Indoor use only
Operating Temperature	10°C to 40°C
Cooling	Convection through metal case
Relative Humidity	60%
Altitude	2,000 m (operating)

Mechanical Characteristics:

Maximum Voltage	Output: 2,500 Volts Peak
Maximum Pulse Length	125 ms @ 500 Volts peak or 5 ms @ 2,500 Volts peak
Pollution Degree 2	(Not to be operated in conductive pollutants atmosphere)

Introduction

The ECM 399 is an electroporation system specifically designed to produce the precise field strengths and pulse lengths required for the transformation of gram -bacteria yeast and limited transfection of mammalian cells.

ECM 399 features include:

- Sophisticated digital design
- High resolution, easy to read Liquid Crystal Display providing readout of: status, voltage mode, setpoint and actual parameters
- Voltage range of 2 to 500 V with 2V resolution in LV mode with a 150 ohm resistance and 1050 μ F capacitance delivering a pulse length of approximately 5 to 55 ms using a low impedance electroporation buffer depending on volume and temperature
- Voltage range of 10 to 2500 V with 10 V resolution in HV mode with a 150 ohm resistor and a 36 μ F capacitor delivering a pulse length of approximately 5 ms using a high impedance electroporation buffer
- Easy three step experimental approach: simply initialize instrument, dial in voltage and mode with the voltage adjust knob, and deliver electroporation pulse by pressing the start switch
- Dual monitoring and display of peak output voltage and time constant to ± 1 percent

Operation: Getting Started

Carefully open the box containing the ECM 399 Electroporation System. Verify receipt of the following items for 45-0050 Electroporator Only:

- ECM 399 Pulse Generator (1)
- Power Cord (1)

BTX Electroporation Systems may be customized with the addition of various electrodes and accessories. The following items complete a typical 45-0000 system order:

- Personal Electroporation Pack (1)
- Model 610 BTX Cuvettes Plus (10)
- Model 620 BTX Cuvettes Plus (10)
- Model 640 BTX Cuvettes Plus (10)

If you have ordered alternative or different items, please verify their receipt.



Fig 1 - Typical ECM 399 45-0000 System Components

Quick Start

Installing

1. Install on Bench or work table
2. Allow 1 to 2 inches of clearance for proper cooling. It is normal for the instrument to be slightly warmer than its operating environment.
3. Choose an outlet that is readily accessible.

Connecting

1. Insert female end of power cord into male power interface on the back panel of the ECM 399.
2. Plug male end of power cord into appropriate electrical outlet.
3. Insert male banana plugs of the Personal Electroporation Pack (PEP) into HV output located on front panel of the ECM 399.

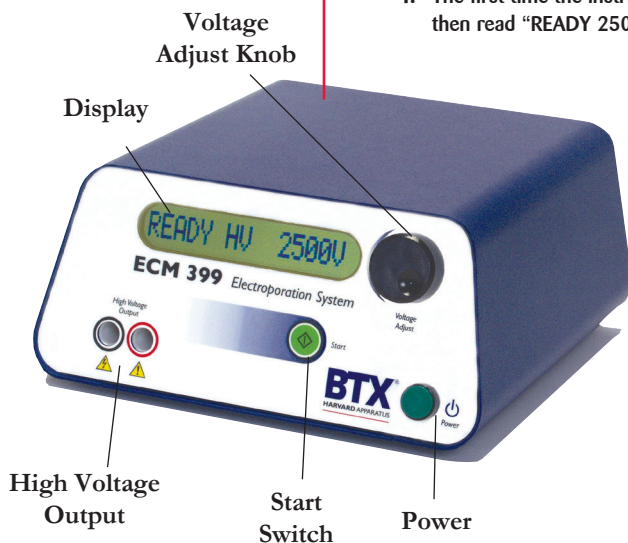
Initializing

1. Push the power switch located on the front panel of the ECM 399.
2. The ECM 399 will go through a series of self-test algorithms to test generator functionality.
3. The display will flash "BTX ECM 399 V1.04" prior to attaining a ready status.
4. The first time the instrument is initialized, the display will then read "READY 2500V HV."

Instrument Controls

Power Switch

1. Switch located on the lower right front panel.
2. Press once to initialize the ECM 399 and once more to turn off.



Quick Start (Continued)

Display

The ECM 399 display will show operational status, voltage mode, and set voltage before delivery of an electroporation pulse. The display will read the peak output voltage delivered and the time constant following the delivery of a pulse.

Status

Ready

Operational and ready to set voltage or deliver a pulse.



Charging

Charging capacitor to the preset voltage level.



Pulsing

Delivering a voltage pulse.



Feedback

Displays peak voltage and time constant.



Sequence Aborted

Aborted delivery of a pulse at the user's request.



SCR Failure

Has not detected an output pulse during the pulsing operation. A short in the electrode could be the cause. This condition can only be reset by turning the power off.



Charge Failure

Failure to charge capacitors properly. Pressing the start switch resets this message.



Setpoint too Low

User has entered setpoint outside of normal operating specifications.



Mode

The ECM 399 will display HV for the high voltage mode and LV for low voltage mode.

Set Voltage

The ECM 399 will display set voltage in 2 volt increments in LV mode, from 2 to 500 V and in 10 volt increments in HV mode from 10 to 2500 V.

Quick Start (Continued)

Actual Parameter Display

The ECM 399 will display the peak discharge voltage (V_p) in V and the time constant (t) in ms. It is normal for the measured voltage to be slightly different than the setpoint. The voltage difference is due to internal resistance and fluctuates with electrical load variations (buffer resistivity).

Voltage Adjust Knob

The ECM 399 Voltage Adjust Knob adjusts the voltage from 2 to 500 V in the LV mode in 2 volt increments and from 10 to 2500 V in the HV mode in 10 volt increments. In the LV mode, increasing of the voltage setpoint past 500 V will result in a switch to the HV mode. The generator will emit a beep signaling a change from the LV mode to the HV mode. The voltage will then readjust to 0 volts, then continue up to 2500 V. In the HV mode, decreasing the voltage setpoint past 0 volts will result in an automatic switch to the LV mode, accompanied by a “beep”. The voltage will then readjust to 500 V. Following the delivery of a pulse, rotate the Voltage Adjust Knob to return to the ready mode. If the voltage adjust knob is set between detentes (notches), the generator may cycle through the actual parameter display following the delivery of a pulse, as if the knob were rotated as above. The voltage adjust knob is speed sensitive. A quick rotation of the knob increases the rate of change of any setpoint.

Note: There are no other steps necessary to change the voltage mode other than adjustment of the Voltage Adjust Knob.

Start Switch

The green Start Switch is activated in the “ready” mode to deliver the electroporation pulse. Once the start switch is activated, the generator will “settle” by bleeding off the capacitors to the preset voltage, prior to delivering the pulse. The maximum settling time is 6 seconds. A pulse sequence may be aborted by pressing the start switch a second time, before delivery of the pulse. Following the delivery of a pulse, press the start switch once to return to the “ready” mode.

- Actual voltage delivered under load will be lower than the set voltage, typically by 1 to 15 %
- Voltage adjust knob controls set voltage AND voltage mode. There is NO separate voltage mode switch!

Operating Basics

Use with the Personal Electroporation Pack (PEP)

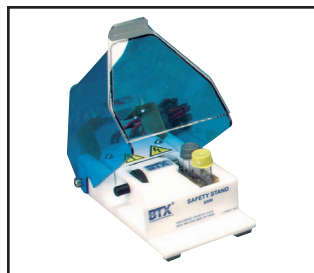
1. Insert the PEP banana plugs into the HV output on the front panel of the ECM 399.
2. Press the power switch to initialize the ECM 399.
3. Verify that the ECM 399 is in the *ready* mode.
4. Dial in the appropriate voltage and mode (LV or HV) with the Voltage Adjust Knob.
5. Verify that the ECM 399 is in the proper voltage (HV or LV) mode.
6. Prepare sample, pipette into the appropriate BTX Disposable Cuvettes Plus, place the cuvette in the PEP and secure with the PEP safety “swing” shield.
7. Press the Start Switch. The ECM 399 will charge and then deliver the electroporation pulse. A beep will indicate that the pulse has been delivered.
8. Process sample. Do not forget to record peak voltage and time constant for documentation purposes.
9. To return to “ready” mode, either rotate the Voltage Adjust Knob or press the Start Switch.
10. To abort a pulse before delivery, press the Start Switch during the “charging” mode.

Use with Alternative Safety Stands 630B

1. Insert the safety stand banana plugs into the HV output on the front panel of the ECM 399.
2. Follow steps 2 to 5 above.
3. Follow the Safety Stand instructions for insertion of the cuvette into the safety stand.
4. Follow steps 7 to 10 above.

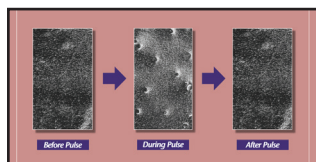


PEP
Item 45-0212

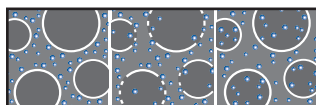


630B
Item 45-0207

Electroporation



Pore Formation



Electroporation Process

Electroporation is the application of controlled, pulsed electric fields to biological systems. If the biological system contains a lipid bilayer, such as is the case if the system is a suspension of cells or liposomes, the pulsed electric field may overcome the field potential of the lipid bilayer, resulting in a reversible breakdown of the bilayer and a resulting formation of temporal pores in the membrane. The pores formed are of the order of 40 to 120 nm. Most pores reseal within a few seconds, after allowing the transfer of materials into and out of the cells.

During a typical electroporation process, target cells and molecules are mixed together. When an electroporation pulse is delivered, the result is the formation of temporal pores. Before the pores reseal, the target molecules are observed to enter the cells. Upon resealing of the pores, the molecules become incorporated within the cell. The eventual target site depends on the application; for example, molecules can remain in the cytoplasm, interact with the membrane, and move into the nucleus.

Applications for electroporation include permeabilization of virtually all cells to a wide variety of molecules and ions. The most common applications for electroporation are the transformation or transfection of cells with DNA or RNA. Other applications for electroporation include electroactivation, electroinsertion of proteins into cell membranes and electroextraction of molecules from cells. Although electroporation has mainly been used as a research tool, recent work has demonstrated its potential for clinical applications. Some areas being explored include:

- electrochemotherapy which involves electroporation for delivering chemotherapeutic agents directly to tumor cells
- encapsulation of drugs/genes into cells for their use as carrier systems
- transdermal delivery of drugs/genes
- gene therapy and delivery of drugs/genes with an electroporation catheter.



Exponential Decay Wave

Electroporation can be characterized by waveform. BTX exponential decay waveform generators, such as the BTX ECM 399 and ECM 630 deliver an exponentially decaying pulse. The length of such a discharge waveform is commonly characterized by the time required for the initial voltage to decay to $1/e$ (roughly $1/3$) of the initial value. To achieve a desired pulse length, appropriate resistance and capacitance must be selected on the instrument. Voltage may be directly set on the instrument.

Applications

Electroporation

Bacteria and Yeast Electroporation

The most common application is transformation. Field strength and pulse length are critical parameters for reporting, optimization, and troubleshooting bacterial and yeast applications.

Mammalian Cell Electroporation

Electroporation has been used successfully to introduce many different molecule types into cells. Most commonly, electroporation is used for the process of transfection, in which nucleic acid (DNA and RNA), is introduced into cells.

Electroporation can be used to deliver oligonucleotides into cells for anti-sense applications. It can be used to deliver proteins into cells, even large enzymes such as restriction enzymes, and antibodies, for various purposes. Peptides have also been electroincorporated. Smaller molecules have been incorporated into cells and liposomes, such as dyes, sugars, and dNTPs.

Electroporation has been used to study cellular activation processes, by electroporabilizing cells to Ca^{2+} , Mg^{2+} , and Na^+ . Electroporation is also used to electroinsert proteins into the cell membrane. Finally, electroporation has been used to introduce drugs, such as the chemotherapeutic agent bleomycin, into cancer cells, in vitro and in vivo.

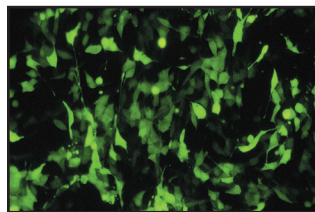
The use of low impedance buffers such as PBS may result in a voltage drop so that the actual peak voltage delivered to samples may be less than the set voltage.

With exponential decay generators, monitoring is necessary to identify the pulse length, or time constant, since this parameter may be very much dependent on the impedance of the sample (sample load).

When using complex and custom electroporation applicators and chambers, the electroporation waveform may be altered and monitoring is again strongly recommended.

Molecules Introduced by Electroporation

- DNA
- RNA
- siRNA
- miRNA
- dNTPs
- Enzymes
- Antibodies
- Other Proteins
- Peptides
- Dyes
- Sugars
- Ions
- Other Molecules



High GFP expression in Mouse PE501

Appendix A: Optimization Strategies

General

The success of electro cell manipulation (ECM) lies in selecting appropriate ECM systems capable of delivering the pulses suitable for the cell being electromanipulated. One, or several pulses of the appropriate field strength, pulse length, and wave shape may be required for this purpose. (Depending on the generator selected.)

The key to success with electroporation-based technologies involves a proper combination of biological, physical, chemical, and pulse parameters. In general, cells must be in mid-logarithmic growth for optimal electroporation. Various temperature regimens have been described. It has been shown that a variety of chemical techniques may increase electroporation efficiencies, including addition of EDTA, DMSO, intracellular salts, and serum before or after the pulse. Optimizing protocols abound. Analysis of these optimization regimens has led to proposals of universal protocols, involving very limited optimization over a narrow range.

Electroporation

1. Vary the voltage in order to vary the field strength, keeping other parameters constant. Assay sample for both viability and endpoint. Plot the field strength versus both viability and endpoint and extrapolate the optimal field strength (voltage divided by gap size) and voltage.
2. Vary the capacitance/resistance/sample volume in order to vary the pulse length (time constant) for exponential decay instruments. Directly vary square wave instrument pulse length. Assay sample for both viability and endpoint. Plot the pulse length versus both viability and endpoint and extrapolate the optimal pulse length/parameters.
3. For multiple pulsing systems/protocols, vary the number of pulses at the optimal field strength kv/cm and pulse length. Assay sample for both viability and endpoint. Plot the number of pulses versus both viability and endpoint, and extrapolate the optimal number of pulses.

Appendix B: Pulse Length Targeting

Choosing A Volume To Obtain A Predetermined Pulse Length For The ECM 399 or ECM 395

Purpose

Derive pulse length curves for the ECM 399 so the user can choose a volume in order to meet a particular pulse length requirement. This Application Note is useful when the researcher wants to reproduce a protocol with a specific pulse length. The 2-step process illustrated below allows the researcher to choose a volume which will provide the desired pulse length.

Definition of Terms

R_s = Sample or chamber resistance.

R_{SYS} = Internal resistance of the ECM 399 or 395.

C_{SYS} = Internal capacitance of the ECM 399 or 395.

T = Time constant or pulse length delivered to sample.

Tools or Parts Needed

Any electrode, any media, ECM 399 or 395, and an optional Enhancer 3000.

Comments

This Application Note is provided so the user can create his/her own plots.

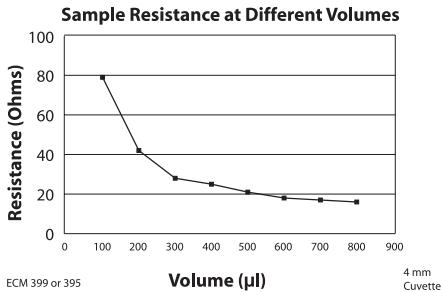
Application

1. Find the resistance of the sample. A quick and easy way to do this is by pulsing the sample once and measuring the pulse length. The resistance of the sample can be calculated with the following equation:

$$R_s = \frac{R_{SYS} * T}{(R_{SYS} * C_{SYS}) * T}$$

Appendix B: Pulse Length Targeting
(Continued)

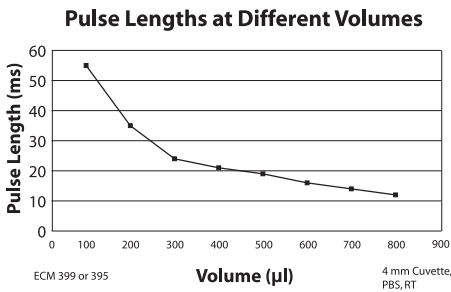
The pulse length may be measured by either the feedback from the ECM 399 or by using the Enhancer 3000. If this is done for different volumes, a sample resistance curve can be constructed.



2. Choose an adequate volume and use the corresponding sample resistance to find the pulse length in milliseconds by the equation below.

$$T = \frac{R_s * R_{sys}}{R_s + R_{sys}} * C_{sys} * 10^{-3}$$

Using different sample resistances a plot of pulse length for each volume can be made.



Appendix C: Electrical Troubleshooting

Instrument does not power up:

Verify that the power cord is fully inserted in the instrument and in the wall outlet. Verify that the fuse is not blown. Disconnect power cord from the instrument before removing the fuse holder. See Maintenance section for removal of fuse.

The ECM 399 is constantly monitoring the parameters of some of its internal circuitry. In the case of a malfunction, one of the following messages will appear on the display. Note the instructions on the following page.

SCR Failure:

The ECM 399 has not detected an output pulse during the pulsing operation. A short in the electrode could be the cause. Unplug the generator for 90 seconds then reset by cycling the power and pulsing one more time. If the same message reappears, call BTX Customer Service.

Charge Failure:

The ECM 399 has detected a failure to charge its capacitors properly. Press the start switch to reset this message. A very low line voltage or a brownout is likely the cause. Verify that the circuit powering the ECM 399 is adequately rated. If the message persists, call BTX Customer Service.

Setpoint too Low:

The user has entered a setpoint that is outside of normal operating specifications. Increase voltage setting.

Appendix D: Experimental Troubleshooting

Arcing

Verify electrical component functionality. Verify properties of cell sample (do cells need to be washed? Is the buffer appropriate for application?). Verify properties of transfectant/molecule (Is the DNA well purified?) Try reducing the voltage or increase sample volume until arcing is no longer a problem.

Low (or no) transfection efficiency, or incorporation

Verify physical, biological, and chemical parameters. Verify delivery of the pulse and pulse parameters. Is the voltage correct? Chamber gap? Pulse length or appropriate instrument settings? Number of pulses? If so, follow Optimization Guidelines outlined in Appendix A.

Low viability

Verify physical, biological, and chemical parameters. Are the voltage, chamber gap, pulse length (time constant), pulse number and other instrument settings correct? If so, reduce voltage, pulse length, or number of pulses and re-optimize protocol to improve viability as outlined in Appendix A.

Appendix E: Glossary of Electrical Terms

Amplitude

The instantaneous value of current or voltage in amperes or volts.

Capacitor

A device that stores electric energy in the form of an internal electric field. Energy is delivered when a current flows out of a capacitor. The current normally follows an exponential curve.

Dielectric

A material that has a high resistivity and can store energy in the form of an electric field.

Direct Current (DC)

Current whose amplitude is constant with time. Direct currents are used to form temporary pores in bi-lipid membranes. Cells may fuse when pores in the membranes of two juxtaposed cells reseal after a DC application.

Divergence

The deviation of electric field lines from a parallel homogeneous condition. A highly divergent field has field lines that rapidly change amplitude (or strength) and direction in the area of interest.

Electric Field

The electric potential difference between two points divided by the distance separating those points. Expressed in volt/cm.

Electric Field Force

The mechanical force acting on any electric charge when placed in an electric field.

Exponential Decay

Non linear waveform typical of capacitor charge and discharge currents and voltages. The exponential decay waveform is characterized by its time constant, the time it takes the voltage to decay to $1/e$ of the peak voltage.

Field Strength

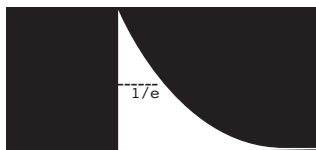
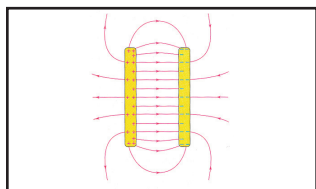
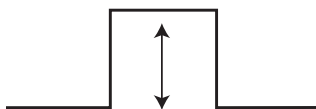
See Electric Field.

Frequency

The number of times an oscillation goes through a complete cycle in one second. The unit is either cycle/sec or Hertz (Hz).

Homogenous Electric Field

An electric field where the direction and strength of the field lines are constant.



Appendix E: Glossary of Electrical Terms (Continued)

Heterogeneous Electric Field

An electric field where the direction and strength of the field lines is varying.

Potential

The difference in volts between two points in space.

Pulse Length

The length of time that an electric signal is present. The end of an exponential pulse is the point at which the waveform has decayed to $1/e$ of the peak value.

Voltage

The difference of electric potential between two points in space. Expressed in volts (V).

Wave Forms

The shape of time varying electric signals. Typically exponential decay, square.



Appendix F: Glossary of Biological & Technical Terms

Dielectric Breakdown

The reversible breakdown of lipid bilayer membranes as a result of the application of a DC electroporation pulse. Sufficiently high field strength may increase the membrane potential past a critical point leading to the breakdown of the membrane.

Electroinsertion

The use of electroporation to insert molecules into lipid bilayer membranes.

Electropermeabilization

The use of electroporation to make cells, protoplasts, or liposomes permeable to ions and small molecules in their extracellular environment.

Electroporation

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

Hydrostatic Pressure

The pressure in liquids at rest.

Lipid Bilayer

An assembly of lipid and protein molecules held together by non-covalent interactions. All biological membranes share this common structure.

Osmotic Pressure

The applied pressure required to prevent the flow of solvents of different concentration across a semipermeable membrane.

Pore

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

Pressure Gradient

The difference in pressure between two points in a medium.

Protoplasts

The plant cell proper, with the cellulose cell wall removed.

Relaxation Time

The time a system requires to reach equilibrium.

Appendix F: Glossary of Biological & Technical Terms (Continued)

Transfection

The introduction of nucleic acids into animal cells. Stable transfections result in integration of nucleic acids into host chromosomes and the inheritance of associated traits in progeny cells. Transient transfections result in temporary expression of exogenous nucleic acids.

Transformation

The introduction of nucleic acids into microorganisms and plant cells.

Turgor Pressure

The pressure in capillaries.

Appendix H: Accessories and Replacement Parts

Catalog No.	Model	Description
45-0000	ECM 399	ECM 399 System
45-0050	ECM 399	ECM 399 Generator only
45-0212	PEP	PEP Personal Electroporation
45-0207	630B	Cuvette Safety Stand, Adjustable Gap
45-0124	Pack, Blue 610	Disposable Electroporation Cuvettes Plus, 1 mm, 50 per bag
45-0125	620	Disposable Electroporation Cuvettes Plus, 2 mm, 50 per bag
45-0126	640	Disposable Electroporation Cuvettes Plus, 4 mm, 50 per bag

Maintenance

The ECM 399 generator requires no special maintenance Other than keeping It clean.

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

Fuse Replacement

Key information

1. Make Sure the power cord is disconnected from the main supply before servicing the fuse.
2. Use only type 5 x 20mm, 2.5 amp, 250 volte type T (time delay) fuses.

Turn off power and remove power cord from power module. Use a straight blade screwdriver and follow instructions in figure below.



- (1) To remove the fuse use screwdriver, push in, and turn to the left.
- (2) Remove fuse from cap and replace with new fuse.
- (3) Return fuse and cap insert into holder push in and turn to the right.

Алматы (727)345-47-04	Иваново (4932)77-34-06	Магнитогорск (3519)55-03-13	Ростов-на-Дону (863)308-18-15	Тольятти (8482)63-91-07
Ангарск (3955)60-70-56	Ижевск (3412)26-03-58	Москва (495)268-04-70	Рязань (4912)46-61-64	Томск (3822)98-41-53
Архангельск (8182)63-90-72	Иркутск (395)279-98-46	Мурманск (8152)59-64-93	Самара (846)206-03-16	Тула (4872)33-79-87
Астрахань (8512)99-46-04	Казань (843)206-01-48	Набережные Челны (8552)20-53-41	Санкт-Петербург (812)309-46-40	Тюмень (3452)66-21-18
Барнаул (3852)73-04-60	Калининград (4012)72-03-81	Нижний Новгород (831)429-08-12	Саратов (845)249-38-78	Ульяновск (8422)24-23-59
Белгород (4722)40-23-64	Калуга (4842)92-23-67	Новокузнецк (3843)20-46-81	Севастополь (8692)22-31-93	Улан-Удэ (3012)59-97-51
Благовещенск (4162)22-76-07	Кемерово (3842)65-04-62	Ноябрьск (3496)41-32-12	Саранск (8342)22-96-24	Уфа (347)229-48-12
Брянск (4832)59-03-52	Киров (8332)68-02-04	Новосибирск (383)227-86-73	Симферополь (3652)67-13-56	Хабаровск (4212)92-98-04
Владивосток (423)249-28-31	Колומна (4966)23-41-49	Омск (3812)21-46-40	Смоленск (4812)29-41-54	Челябинск (351)202-03-61
Владикавказ (8672)28-90-48	Кострома (4942)77-07-48	Орел (4862)44-53-42	Сочи (862)225-72-31	Череповец (8202)49-02-64
Владимир (4922)49-43-18	Краснодар (861)203-40-90	Оренбург (3532)37-68-04	Ставрополь (8652)20-65-13	Чита (3022)38-34-83
Волгоград (844)278-03-48	Красноярск (391)204-63-61	Пenza (8412)22-31-16	Сургут (3462)77-98-35	Якутск (4112)23-90-97
Вологда (8172)26-41-59	Курск (4712)77-13-04	Петрозаводск (8142)55-98-37	Сыктывкар (8212)25-95-17	Ярославль (4852)69-52-93
Воронеж (473)204-51-73	Курган (3522)50-90-47	Псков (8112)59-10-37	Тамбов (4752)50-40-97	
Екатеринбург (343)384-55-89	Липецк (4742)52-20-81	Пермь (342)205-81-47	Тверь (4822)63-31-35	

Россия +7(495)268-04-70 **Казахстан** +7(727)345-47-04 **Беларусь** + (375)257-127-884 **Узбекистан** +998(71)205-18-59 **Киргизия** +996(312)96-26-47

эл. почта: bxt@nt-rt.ru || сайт: <https://btx.nt-rt.ru/>