Ingenio® EZporator® Electroporation System

Product User Manual

MIR 51000

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RevB 121520

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Technology Overview

Electroporation is a physical transfection method that utilizes short electrical pulses to create transient membrane pores in cells through which small particles (e.g. DNA, RNA, proteins, etc.) can pass. Optimal electroporator pulse settings and composition of cell resuspension solution greatly enhance electro-permeabilization and promote passage of particles through the cell membrane. Since electroporation is an effective transfection method regardless of the type of molecule delivered or the target cells, this technique provides a robust and universal approach for multiple applications, including gene expression, RNAi, stable cell line generation and CRISPR/Cas genome editing.

Electroporation is an invaluable alternative for cell types that are not responsive to chemical transfection and often yields significantly higher transfection efficiency. Optimizing parameters such as nucleic acid amount, cell density and voltage for each cell type will improve transfection efficiency and decrease cell mortality, thus making electroporation suitable for transfecting various cell types with any type of nucleic acid or small molecule.

The Ingenio® EZporator® Electroporation System was developed with both efficiency and simplicity for the researcher in mind. This system utilizes an *exponential decay pulse** to effectively deliver cargo to cells in a cuvette with the push of a button. The Ingenio® EZporator® was designed for use with Ingenio® Electroporation Kits and Solution. Use of electroporation solutions not manufactured by Mirus Bio may result in low transfection efficiency and high cellular cytotoxicity. Additionally, use of in-compatible electroporation solutions may cause damage to the instrument and will void the warranty. More information on Ingenio® Electroporation Solution and Kits is available at mirusbio.com/ingenio.

*In exponential-decay pulse conditions, a set voltage is released from the capacitor and decays exponentially. See Optimization Section (page 14) for additional details.

Ingenio® EZporator® Electroporation System and Ingenio® Electroporation Solution, cuvettes and cell droppers.

System Components and Features

The EZporator® Electroporation System was designed for mammalian and insect cell transfection with Ingenio® Electroporation Kits and Solution.

System features include:

- Digital Liquid Crystal Display (LCD) user interface
- Pulse Generator to deliver exponential decay pulse at user-determined voltage. NOTE: In exponential decay pulse conditions, a set voltage is released from the capacitor and decays exponentially.
- Cuvette Chamber compatible with 0.1 cm, 0.2 cm or 0.4 cm cuvettes
- Power cords with Type B, F and G electrical outlet plugs
- Low Voltage (LV) mode
  - Voltage range of 20 to 400 V with 2 V resolution
  - 150 Ω internal resistance and 1,050 µF capacitance
- High Voltage (HV) mode
  - Voltage range of 30 to 2,500 V with 10 V resolution
  - 150 Ω internal resistance and 36 µF capacitance
- Streamlined experimental approach: (1) Initialize instrument, (2) Set voltage and mode with the Voltage Adjust knob and (3) Deliver electroporation pulse by pressing the Pulse button.
- Dual monitoring and display of peak output voltage and time constant

System Components:
Upon receipt, carefully open the box containing the EZporator® Electroporation System and verify that the box contains the following:

- EZporator® Pulse Generator (1)
- EZporator® Cuvette Chamber (1)
- Power cords (3)

EZporator® Pulse Generator

EZporator® Cuvette Chamber

Power Cords

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Technical Specifications

Display	Type: 16-character LCD backlit
Dimensions (W×D×H)	254 mm × 254 mm × 140 mm (Pulse Generator)
Power Source	
Voltage	100 to 240 VAC, 50-60 Hz, CAT I
Power	800 W
Fusing	2.5 A, T rating 250 V
Environmental Usage:	
Operating Temperature	10°C to 40°C (NOTE: Intended for indoor use only)
Cooling	Convection through metal case
Relative Humidity	Avoid excessively humid working environments
Altitude	< 2,000 m above sea level (operating)
Mechanical Characteristics:	
Maximum Voltage	Output: 2,500 V peak
Maximum Pulse Length	125 ms at 400 V peak or 5 ms at 2,500 V peak
Pulse Waveform	Exponential decay
Resistance	150 Ω internal resistor
Pollution Degree 2	Not to be operated in conductive pollutants atmosphere

Ordering Information:

Catalog #	**Product**
MIR 51000	Ingenio® EZporator® Electroporation System
MIR 51100	Ingenio® EZporator® Pulse Generator
MIR 51200	Ingenio® EZporator® Cuvette Chamber

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General Safety Summary

The EZporator® Electroporation System is safe to use when operated in accordance with this manual. Please read the following safety precautions to ensure proper use, as this system is designed to deliver high voltage electrical pulses. If the equipment is improperly used, the protection provided by the equipment may be impaired.

To prevent hazard or injury, take the following precautions:

- **USE PROPER POWER CORD:** Use only the power cord specified for this product and certified for the country of use.
- **USE PROPER FUSE:** Use only 5 mm × 20 mm, 2.5 A, 250 V type T (time delay) fuses with this product.
- **ORIENT THE EQUIPMENT PROPERLY:** Do not orient the equipment such that it is difficult to manage the connection and disconnection of devices.
- **ENSURE PROPER VENTILATION**: Allow 3 to 5 cm of clearance on all sides of the module for proper cooling.
- **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, ensure that the EZporator® is properly grounded.
- **OBSERVE ALL TERMINAL RATINGS:** Review the user manual to learn the ratings on all connections.
- **AVOID CONTACT WITH INTERNAL CIRCUITRY:** Do not open the product or touch any electronic circuitry inside of the product.
- **AVOID EXPOSURE TO EXCESS MOISTURE:** Do not expose the device to a humidified environment. If fluid is spilled on or near the EZporator® instrument, performance and safety may be compromised.
- **DO NOT OPERATE IF DAMAGED:** If damage is suspected on or to the EZporator® Pulse Generator or Cuvette Chamber, contact qualified service personnel to perform an inspection prior to use.
- **OBSERVE ALL WARNING LABELS ON PRODUCT:** Read all labels on product to ensure proper usage.
- **ENVIRONMENTAL USAGE:** This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen or nitrous oxide.

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General Safety Summary (Continued)

High Voltage Risk

This symbol indicates a risk of electric shock. Electric shocks are dangerous and may cause personal injury or death.

This instrument contains a high voltage power supply adjustable to 2,500 V. This presents a serious risk of personal injury if not used in accordance with design and/or use specifications, if used in applications or with products for which they are not intended or designed, or if they are used by untrained or unqualified personnel.

Please take special note of the following:

- The user must read this manual carefully before operating the instrument.
- Removing the EZporator® Pulse Generator cover and breaking the "Warranty Void if Seal Broken" sticker will void the warranty.
- Power off the instrument before connecting or disconnecting any cords/cables.
- Do not open the Cuvette Chamber or attempt to touch the electrodes while the electrical pulse is being applied.
- All service must be performed by Mirus Bio authorized personnel only.

If there are any questions about the operation of this instrument, call Mirus Bio Technical Support at 888.530.0801 or +1.608.441.2852.

Caution Notice

This symbol indicates that caution is advised. Proper set-up, usage and PPE when using this instrument are required.

The Ingenio® EZporator® Electroporation System is intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) intended for laboratory equipment applications as well as the applicable safety requirements for electrical equipment for measurement, control and laboratory use. The unit itself does not generate waste, but may be used to treat samples that are hazardous. Please use appropriate PPE and ensure disposal in accordance with local regulations and practices.

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

This product is for RESEARCH USE ONLY and is not intended for clinical use on animals or human patients.

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Set-up Instructions

Installation

Place the EZporator® Pulse Generator on a laboratory bench or work table. Be sure to allow 3 to 5 cm of clearance on all sides of the module for proper cooling. When selecting a workspace, choose a stable, dry location with an easily accessible power outlet. NOTE: It is normal for the EZporator® Pulse Generator module to be slightly warmer than its operating environment.

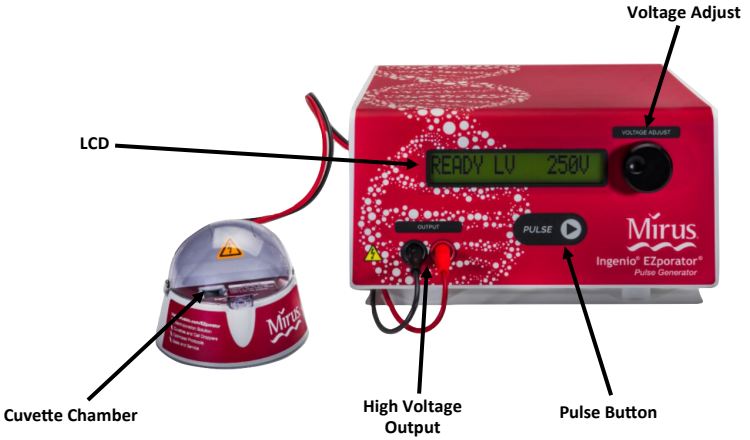
Connect the power cord to the EZporator® Pulse Generator module AC Input on the back panel, then insert the power cord plug into an appropriate electrical outlet (i.e. 110 - 240 VAC).

To connect the EZporator® Cuvette Chamber to the Pulse Generator, insert the color coded plugs attached to the Cuvette Chamber into the HV Output located on front panel of the EZporator® Pulse Generator.

Initializing

1. Turn the EZporator® Pulse Generator on by pushing the green power button located at the back of the instrument (see page 19). NOTE: Push the power button once to turn on. Push again to turn off. Do not hold the power button.
2. The EZporator® will go through a series of self-test algorithms to test generator functionality. The display will flash "EZporator®" and the software version (e.g. V1.01) prior to attaining a ready status.
3. When the instrument is initialized for the first time, the display will read "READY LV 400V."

Instrument Features and Controls:



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Set-up Instructions (Continued)

Display

Once initialized, the EZporator® Pulse Generator display will show the operational status, voltage mode (i.e. LV or HV) and set voltage. Once a pulse is delivered to the cuvette containing cells, the display will read the peak output voltage delivered and the time constant.

Status options include:

Ready Mode

The EZporator® is operational and ready to deliver a pulse. The LCD display will show HV for the high voltage mode and LV for low voltage mode. NOTE: Ensure that the correct voltage mode is selected for your cell type and electroporation buffer. *Use LV mode for mammalian cell types in Ingenio® Solution.*

- LV mode: from 20 to 400 V (2 V increments), 1050 µF capacitance
- HV mode: from 20 to 2500 V (10 V increments), 36 µF capacitance

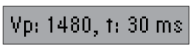
Initializing and Charging

Initializing and charging capacitor to the preset voltage level.

Pulsing

Delivering a voltage pulse.

Feedback



Displays peak voltage and time constant for delivered pulse.

Sequence Aborted

Aborted delivery of a pulse at the user's request. To abort a pulse sequence, press the Pulse Button a second time before delivery of the pulse.

SCR Failure

The instrument has not detected an output pulse during the pulsing operation. As a safety precaution, this message can only be reset by turning the power off.

Charge Failure

The capacitors failed to charge properly. To reset this message, press the 'Pulse' button.

Setpoint Too Low

The entered voltage setpoint is below the normal operating specifications. No pulse has been applied.

Set-up Instructions (Continued)

Post-pulse Display

The EZporator® instrument will display the peak discharge voltage (V_P) in volts (V) and the time constant (t) in milliseconds (ms). It is normal for the measured voltage to differ slightly from the setpoint. This is due to internal resistance and will fluctuate with electrical load variations (i.e. buffer conductivity). Following the delivery of a pulse, press the Pulse Button or rotate the Voltage Adjust knob to return to the ready mode.

NOTE: With exponential decay generators such as the EZporator®, it is necessary to identify and monitor the time constant (t), as this variable is dependent on the conductivity and osmolarity of the electroporated sample. Significant changes in the electroporation sample (e.g. variations in cell density, volume of nucleic acid added, electroporation solution used) will alter the time constant.

Voltage Adjust Knob

The EZporator® Voltage Adjust knob controls set voltage AND voltage mode (i.e. LV and HV modes). There is no separate voltage mode switch. Use the Voltage Adjust knob to adjust the voltage from 20 to 400 V in LV mode (in 2 V increments) and from 30 to 2,500 V in HV mode (in 10 V increments). If in LV mode, increasing of the voltage setpoint past 400 V will result in a switch to HV mode. The voltage then readjusts to 0 V and continues up to 2,500 V. If in HV mode, decreasing the voltage setpoint past 0 V will result in a switch to LV mode. The voltage then readjusts to 400 V.

NOTE 1: The EZporator® emits a sound when switching between LV and HV modes.

NOTE 2: The Voltage Adjust knob is speed sensitive. A quick rotation increases the rate of change of the voltage setpoint.

NOTE 3: LV mode is used for mammalian and insect cell types.

Pulse Button

The Pulse button is activated in 'READY' mode. Once pressed, the generator will bleed off the capacitors to the preset voltage, prior to delivering the pulse. The capacitor bleed time is typically between 1-6 seconds. A pulse sequence may be aborted by pressing the Pulse button a second time before delivery of the pulse. Following the delivery of a pulse, press the Pulse button or rotate the Voltage Adjust knob to return to the 'READY' mode.

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EZporator® System Protocols

Quick Reference Guidelines

1. Connect the EZporator® Cuvette Chamber to the Pulse Generator instrument by inserting the black and red color-coded leads of the Cuvette Chamber into the Output ports on the front panel of the Pulse Generator.
2. Press the power switch on the back panel to initialize the EZporator®.
3. Prepare samples by combining target cells and molecules in an electroporation buffer. Pipette an appropriate volume of the prepared sample into a 0.1 cm, 0.2 cm or 0.4 cm cuvette.

   NOTE: We recommend using the Ingenio® Electroporation Solution and Kits for mammalian and insect cell electroporations.
4. Verify that the EZporator® is in 'READY' mode. Then use the Voltage Adjust knob to dial in the appropriate voltage and mode (LV or HV) for the cell type and buffer to be electroporated. NOTE: LV mode is used for mammalian and insect cell types.
5. Place the cuvette in the Cuvette Chamber and close the safety dome.
6. Press the Pulse button. The EZporator® will charge and then deliver the electroporation pulse. A sound will indicate that the pulse was delivered. NOTE: To abort a pulse, press the Pulse button during the 'CHARGING' interval before the pulse is delivered.
7. Record the peak voltage (Vp) and time constant (t).
8. Process the sample as required for the experiment.
9. Following the delivery of a pulse, press the Pulse button or rotate the Voltage Adjust knob to return to the 'READY' mode.
10. Repeat steps 5-9 for additional samples.

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EZporator® System Protocols (Continued)

Instructions for Use with Ingenio® Electroporation Solution and Kits

The Ingenio® Electroporation Solution and Kits provide a universal, high efficiency, low toxicity solution for electroporating nucleic acids (i.e. DNA, RNA, oligonucleotides, etc.) or other molecules into mammalian and insect cell types.

Transient plasmid DNA or siRNA electroporation protocol

A. Plate cells

1. Approximately 18-24 hours before electroporation, passage cells to attain an optimal cell density at the time of electroporation (i.e. 70 - 90% confluent for most cell types).
   For adherent cells: Plate cells at a density of 0.8 - 3.0 × 10^5 cells/ml
   For suspension cells: Seed cells at a density of 1 - 2 × 10^6 cells/ml
2. Incubate cell cultures overnight.

B. Prepare Ingenio® Solution/nucleic acid/cell mixture for electroporation (immediately before electroporation)

1. Warm Ingenio® Electroporation Solution, trypsin-EDTA (if applicable) and complete growth medium to room temperature.
2. Harvest and count cells to determine cell density (cells/ml).
3. Determine the total electroporation volume required to perform the desired number of electroporations.
   For 0.2 cm cuvettes: Multiply number of electroporations by 0.1 ml
   For 0.4 cm cuvettes: Multiply number of electroporations by 0.25 ml
4. Calculate the cell volume required for all electroporations:

$$\text{Cell Volume (ml)} = \frac{\text{Final Cell Density (cells/ml)} \times \text{Volume from Step B3 (ml)}}{\text{Harvested Cell Density from Step B2 (cells/ml)}}$$

   For adherent cells: Use a final cell density of 1 - 5 × 10^6 cells/ml
   For suspension cells: Use a final cell density of 10 × 10^6 cells/ml
5. Pipette the cell volume (from step B4) of harvested cells into a new tube and centrifuge at 300 *× g* for 5 minutes. Aspirate the supernatant.
6. During centrifugation, add pre-warmed complete culture medium to a new culture dish to accept cells following electroporation.
7. After centrifugation, resuspend cells in Ingenio® Electroporation Solution using the volume determined in step B3.

(Cont. on next page)

EZporator® System Protocols (Continued)

8. Add the desired amount of nucleic acid to the Ingenio® Electroporation Solution and cell mixture. Mix gently but thoroughly. Do not create air bubbles.
   For DNA: Use 20 µg DNA per 1 ml of cells as a starting point.
   For siRNA: Use 250 nM siRNA (final concentration) as a starting point.
   NOTE: For further optimization, refer to the Ingenio® Full Protocol.

C. Perform Electroporation

1. Aliquot Ingenio® Solution/nucleic acid/cell mixture into cuvettes for electroporation.
   For 0.2 cm cuvettes: Pipet 100 µl total mixture per cuvette
   For 0.4 cm cuvettes: Pipet 250 µl total mixture per cuvette
2. Electroporate the cells at room temperature.
   NOTE: The optimal pulse conditions or program settings will vary depending on the cell type and electroporator used. For most mammalian and insect cell types, optimal conditions fall within the following ranges in LV mode:
   For 0.2 cm cuvettes: 80-160 V (voltage) and 800-1000 µF (capacitance)
   For 0.4 cm cuvettes: 200-300 V (voltage) and 800-1000 µF (capacitance)
3. Immediately transfer the electroporated cells into the prepared culture dish (from step B6). NOTE: The optimal post-electroporation cell culture density will depend on the cell type, transfected nucleic acid and post-electroporation incubation period.
4. Incubate the electroporated cells in appropriate culture medium for 12-72 hours or as required. A culture medium change may be necessary for longer incubations.
5. Harvest cells and assay as required.

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Optimization

Electroporation parameters (e.g. cell density, nucleic acid amount, voltage) should be optimized for each cell type to ensure best possible transfection efficiency and cell viability. The suggestions below yield high efficiency electroporation of most mammalian and insect cell types.

Cell Density and Passage Number

- **Cell division.** Passage cultures approximately 18-24 hours before electroporation to ensure that cells are actively dividing and reach the appropriate density (i.e. 70-90% confluent for adherent cells or 2 - 4 × 10^6 cells/ml for suspension cells) at the time of harvesting for electroporation. In general, cells should be in mid-logarithmic growth for optimal electroporation.
- **Cell density at electroporation.** Determine the optimal cell density for each cell type to maximize electroporation efficiency. The optimal cell density for electroporation is typically between 1 - 10 × 10^6 cells/ml. Higher cell densities are typically recommended for suspension cells (e.g. 1 x 10^7 cells/ml), whereas the recommended range for adherent cells is 1 - 5 × 10^6 cells/ml.
- **Cell passage number.** Use of very low or very high passage cells may affect experimental results. Use cells of similar passage number for experimental reproducibility.

Nucleic Acid Purity and Concentration

- **Plasmid DNA.** Use highly purified, sterile and contaminant-free DNA for electroporation. Plasmid DNA preparations that are endotoxin-free and have A260/280 absorbance ratio of 1.8-2.0 are desirable. DO NOT use DNA prepared using miniprep kits or DNA that is less than 0.5 mg/ml in concentration. To determine the best plasmid DNA concentration for electroporation, try DNA concentrations in the range of 5-50 µg/ml of final electroporation volume.
- **siRNA.** Use siRNA that is highly pure, sterile and the correct sequence. Try siRNA in the range of 250-750 nM (final concentration in cuvette) to determine the best siRNA concentration for electroporation.

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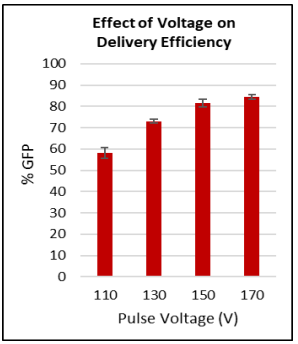
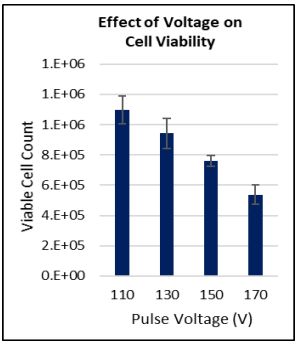
Optimization

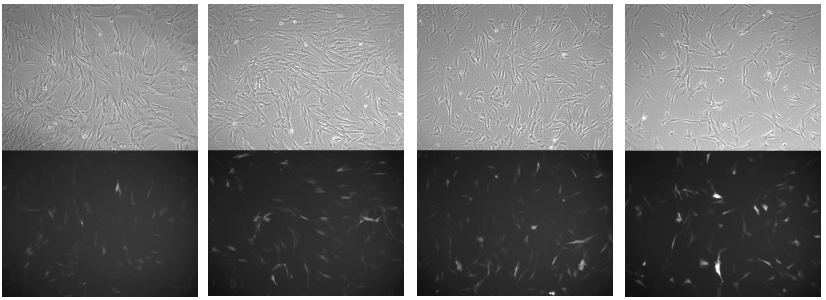
Pulse Conditions

- **Voltage.** For cells resuspended in Ingenio® Electroporation Solution, the exponential decay pulse conditions for most cell types fall within a voltage range of 80-160 V when using 0.2 cm cuvettes (100 µl volume) and 200-300 V when using 0.4 cm cuvettes (250 µl volume). To optimize electroporations, vary the voltage in 10 V increments (starting at 200 V for 0.4 cm cuvettes and 80 V for 0.2 cm cuvettes).
- **Time Constant.** Time constant (TC) values will vary depending on electroporation buffer conductivity, volume in the cuvette, cell density and temperature. Typical TC values with Ingenio® Electroporation Solution range from 19-23 ms in 0.2 cm cuvettes (100 µl volume) and 29-32 ms in 0.4 cm cuvettes (250 µl volume).

Example Voltage Titration: BJ Fibroblasts were electroporated with a GFP-encoding plasmid DNA at the specified voltages in 0.2 cm cuvettes (100 µl volume). Delivery efficiency and cell viability were determined by flow cytometry 48 hours post-electroporation:







110 V **130 V** **150 V** **170 V**

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Pulse Conditions for Common Cell Types

Cell Type	Cuvette Size (cm)	Cell Density ($\times 10^6$ cells/ml)	DNA (µg)	Electroporation Volume (µl)	Voltage (V)
Primary Human Keratinocyte	0.2 0.4	2	2 5	100 250	150 220
Primary MEF	0.2 0.4	5	2 5	100 250	150 230
Primary Rat Cortical Neuron	0.2 0.4	1	2 -	100 -	120 -
A549	0.2 0.4	5	- 5	- 250	- 280
BHK21	0.2 0.4	10	2 5	100 250	150 280
CHO-K1	0.2 0.4	5	2 5	100 250	150 280
COS-7	0.2 0.4	5	2 5	100 250	150 260
HEK 293	0.2 0.4	5	2 5	100 250	160 250
HEK 293T	0.2 0.4	5	- 5	- 250	- 250
HeLa	0.2 0.4	3	2 5	100 250	130 260
Hepa	0.2 0.4	5	2 -	100 -	160 -
HepG2	0.2 0.4	5	2 5	100 250	170 250
HL-60	0.2 0.4	10	2 5	100 250	150 276
HUVEC	0.2 0.4	3	- 5	- 250	- 250
Jurkat	0.2 0.4	10	2 5	100 250	150 260
K562	0.2 0.4	10	2 5	100 250	130 250
MCF-7	0.2 0.4	3	2 -	100 -	150 -
NIH-3T3	0.2 0.4	10	2 5	100 250	160 260
NIKS	0.2 0.4	2	2 5	100 250	170 280
PC-12	0.2 0.4	3	2 5	100 250	130 240
RAW 264.7	0.2 0.4	5	2 5	100 250	150 260
SH-SY5Y	0.2 0.4	5	- 5	- 250	- 250
SK-BR-3	0.2 0.4	5	2 5	100 250	160 260
SK-N-MC	0.2 0.4	5	2 5	100 250	90 240
THP-1	0.2 0.4	10	2 5	100 250	140 250
U-937	0.2 0.4	10	- 5	- 250	- 260
Vero	0.2 0.4	5	2 -	100 -	170 -
Other (Starting Point)	0.2 0.4	5-10	2 5	100 250	80-160 200-300

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Instrument Troubleshooting

Instrument does not power up: Verify that the power cord is fully inserted in the instrument and wall outlet. To verify that the fuse is not blown, first disconnect the power cord from the instrument and then remove the fuse holder. See Maintenance section (page 20) for fuse replacement instructions.

Error messages appear: The EZporator® System is constantly monitoring the parameters of some of its internal circuitry. In the case of a failed pulse, one of the following messages will appear on the display:

SCR failure: The EZporator® 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, contact Mirus Technical Support.

Charge failure: The EZporator® has detected a failure to charge its capacitors properly. Press the 'Pulse' button to reset this message. A very low line voltage or a brownout may be the cause. Verify that the outlet powering the EZporator® is adequately rated. If the message persists, contact Mirus Technical Support.

Setpoint too low: The user has entered a setpoint that is outside of normal operating specifications. Increase the voltage setting.

NOTE: Although the instrument firmware allows input voltages down to 2 V in LV mode and 10 V in HV mode, these are below the recommended voltage settings. "Setpoint too low" or "SCR failure" error messages are likely to occur in this very low voltage range because of the extended time needed to discharge capacitor banks down to < 20 V. At ≥ 20 V, the instrument will reliably discharge and send pulses.

Arc discharge: Arcing is a complete or partial electrical discharge circumventing the sample and is often accompanied by a spark-like flash and sound. Arcing may be caused by a faulty cuvette or inappropriate voltage setpoint for the solution used or volume in the cuvette. Ensure that cells were properly resuspended in Ingenio® Electroporation Solution and that nucleic acids are highly pure. Consider reducing the voltage setpoint or increasing sample volume until arcing is no longer a problem.

NOTE: There is no error message for arcing: however, the electroporated cell population is unlikely to be viable should arcing occur.

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Experimental Troubleshooting: Low Electroporation Efficiency

Problem	Solution
Cell density not optimal at time of electroporation	Determine the best cell density for each cell type to maximize electroporation efficiency. For most suspension cells, a cell density of 1×10^7 cells/ml is recommended for electroporation. For adherent cells, a range of $1 - 5 \times 10^6$ cells/ml is recommended. Use of higher or lower densities may increase cell viability depending on cell type.
Cells not actively dividing at the time of electroporation	Passage cells at least 18–24 hours before electroporation to ensure that the cells are actively dividing and reach optimal cell density at the time of electroporation.
Suboptimal DNA concentration	Confirm DNA concentration and purity. Use plasmid DNA preps that have an $A_{260/280}$ absorbance ratio of 1.8-2.0. The optimal DNA concentration generally ranges between 5-50 µg/ml of final electroporation volume. Start with 20 µg/ml of total electroporation volume.
Low quality plasmid DNA	Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection. Do not use DNA prepared using miniprep kits as it might contain high levels of endotoxin.
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of the plasmid DNA.
Proper experimental controls were not included for plasmid delivery	To verify efficient electroporation, deliver a positive control such as a luciferase or green fluorescent protein (GFP) encoding plasmid. To assess delivery efficiency of plasmid DNA, use Mirus Bio *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kit to label the target plasmid **or** use Mirus Bio prelabeled *Label* IT® Plasmid Delivery Controls.
Suboptimal siRNA concentration	The optimal siRNA concentration generally ranges between 250-750 nM final concentration. Use 250 nM siRNA as a starting point.
Incorrect siRNA sequence	Ensure that the sequence of the siRNA is correct for the gene of interest. More than one sequence may need to be tested for optimal knockdown efficiency and to ensure proper targeting.
Poor quality of siRNA	Avoid siRNA degradation by using RNase-free handling procedures and plastic ware. Degradation of siRNA can be detected on acrylamide gels.
Proper controls were not included for siRNA delivery	Recommended controls include: (1) Cells alone, (2) Serum-free medium + Ingenio® Electroporation Solution + a non-targeting siRNA. To verify efficient transfection and knockdown, deliver a siRNA targeted against a ubiquitous gene, e.g. GAPDH or Lamin A/C, followed by target western blotting or mRNA quantification.
Electroporation incubation time	Determine the optimal electroporation incubation time for each cell type and experiment. Test a range of incubation times (e.g. 12-72 hours). The best incubation time is generally 24-48 hours.

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Experimental Troubleshooting: High Cellular Toxicity

Problem	Solution
Voltage setpoint too high for cell type	Decrease the voltage by increments of 10 V until viability improves.
Cells not transferred immediately to culture vessel containing complete growth medium	Transfer the cells from each cuvette to a culture dish containing warm complete culture medium immediately after each electroporation.
Endotoxin-contaminated plasmid DNA	Use highly purified, sterile, endotoxin and contaminant-free DNA for electroporation. We recommend using Mirus Bio MiraCLEAN® Endotoxin Removal Kit (MIR 5900) for removal of any traces of endotoxin from your DNA preparation. Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells. DO NOT use DNA prepared using miniprep kits as it might contain high levels of endotoxin.
DNA preparation has too much salt	If DNA was prepared using an ion exchange column with a final ethanol precipitation step, we recommend exchanging the DNA solution to a salt-free or low salt solution, e.g. elute the DNA in water and add 5 mM NaCl.
Excessive amounts of DNA in the final electroporation mix	Reduce the amount of DNA used for electroporation. DNA concentrations as low as 5 µg/ml of the final electroporation volume can be used. Compare toxicity levels against a cells + Ingenio® Electroporation Solution control to assess the effects of the DNA transfected. If you still see toxicity, it is likely due to the cell mixture being too concentrated or presence of too many lysed cells.
Expressed target gene is toxic to cells	Compare toxicity levels against a cells-alone control and cells electroporated with an empty vector to assess the cytotoxic effects of the target protein being expressed. If a lower level of target gene expression is desired in your electroporation experiments, consider reducing the amount of target plasmid. If necessary, maintain the optimal DNA concentration (20 µg) by using carrier DNA such as an empty cloning vector.
Knockdown of an essential gene	If the electroporated siRNA is directed against a gene that is essential to the cell, cytotoxicity may be observed due to knockdown of the target gene. Include a control with non-targeting siRNA to compare the cytotoxic effects of the gene being knocked down.
Cell morphology has changed	Mycoplasma contamination can alter cell morphology and affect electroporation efficiency. Check your cells for Mycoplasma contamination. Use a fresh frozen stock of cells or use appropriate antibiotics to eliminate Mycoplasma. A high or low cell passage number can make cells more sensitive and refractory to electroporation. Maintain a similar passage number between experiments to ensure reproducibility.

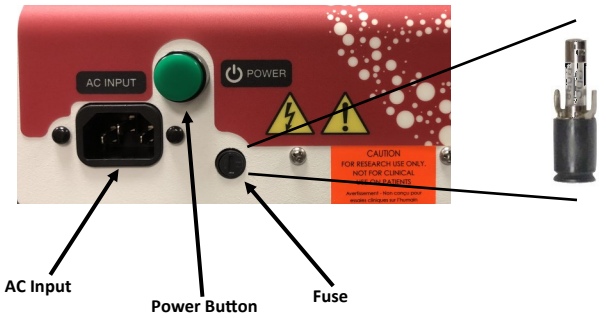
Maintenance

The Ingenio® EZporator® Electroporation System does not need to be calibrated and requires no special maintenance beyond occasional cleaning. To clean, first power off the EZporator® Pulse Generator module and remove the power cord. Carefully clean exterior surfaces using a lint-free cloth and avoid scratching the LCD display window. Dampen (but do not soak) a soft cloth with an aqueous solution of 70-80% isopropyl alcohol or a mild detergent for more thorough cleaning. Do not expose the cuvette chamber to excess moisture.

Fuse Replacement

The EZporator® fuse is located at the back of the instrument, offset below the green Power Button (see below). Blown fuses can easily be replaced by following these instructions:

1. Power off the Pulse Generator and disconnect the power cord from the module before servicing the fuse.
2. Locate the fuse (see below) and insert a small flat-blade screwdriver into the slot on the exterior portion of the fuse cap. Push in and turn left to unlock and remove the fuse cap and fuse.
3. Remove the blown fuse from the cap and replace with a new fuse. NOTE: Use only 5 mm x 20 mm, 2.5 A, 250 V type T (time delay) fuses.
4. Return the fuse and cap to the Pulse Generator module. Carefully turn right with the screwdriver to tighten.



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Warranty

General Warranty

The Ingenio® EZporator® Electroporation System is warrantied for one year from the date of purchase. At its discretion, Mirus Bio will repair or replace the unit if it is found to be defective as to workmanship or materials. This warranty is considered void for any instrument which has been:

1. Subjected to misuse, neglect, accident or abuse
2. Repaired or altered by anyone other than Mirus Bio without express and prior approval
3. Used in violation of instructions furnished by Mirus Bio
4. Changed in any way from its original factory design
5. Used with parts, connections or electrodes not manufactured by Mirus Bio

This warranty extends only to the original purchaser. MIRUS BIO IS NOT LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some territories 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 territories 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, Mirus Bio 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.

FOR U.S. CUSTOMERS: If a defect arises within the warranty period, promptly contact Mirus Bio LLC, 5602 Research Park Blvd., Ste. 210, Madison, Wisconsin, 53719 using our toll free number 888.530.0801 (U.S. Only) or +1-608.441.2852. E-mail: techsupport@mirusbio.com. Goods will not be accepted for return unless a Returned Materials Authorization (RMA) number has been issued by our customer service department. The customer may be 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 or for 90 days, whichever is longer. EZporator® Electroporation System serial numbers are found on the stickers applied to the outer shipping box and on the bottom of the EZporator® Pulse Generator.

FOR CUSTOMERS OUTSIDE THE U.S.: Please contact your official Mirus Bio EZporator® Electroporation System distributor immediately to address any defects that arise within the warranty period.

Warranty (Continued)

Out of Warranty Service

FOR U.S. CUSTOMERS: Phone and email support are provided 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 of your EZporator® Device to Mirus Bio 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 Mirus Bio technicians.

FOR CUSTOMERS OUTSIDE THE U.S.: Please contact your official Mirus Bio EZporator® Electroporation System distributor to address any defects that arise Out of Warranty.

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Notes

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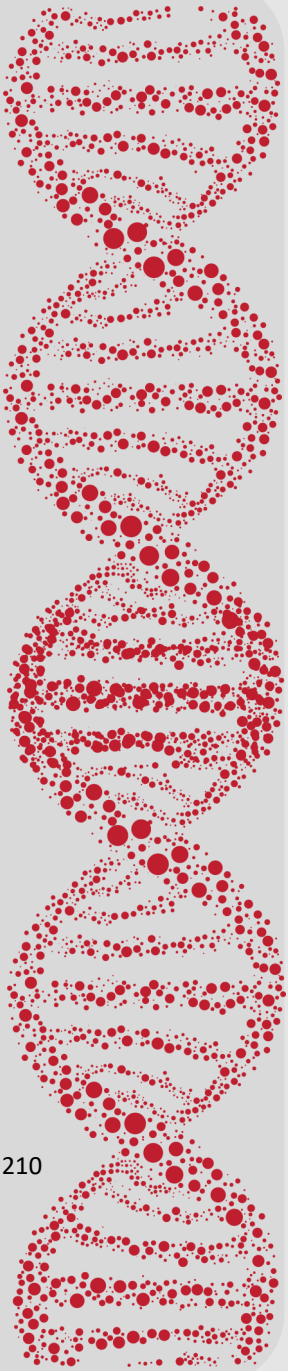

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