Ingenio™ Electroporation Kits & Solution

Protocol for Product Nos. MIR 50111, 50112, 50113, 50114, 50115, 50116, 50117, 50118, 50119

Mirus
It All Begins at the Bench

INTRODUCTION

Ingenio™ Electroporation Kits and Solution provide a universal, high efficiency, low toxicity solution for electroporation of DNA into a broad range of hard to transfect cells. The Ingenio Electroporation Solution is compatible with any electroporation instrument.

SPECIFICATIONS

Storage	Store the Ingenio Electroporation Solution at 4°C. All other materials can be stored at room temperature.
Stability	6 months from the date of purchase, when properly stored and handled.
Number of Electroporations	One ml of Ingenio Electroporation Solution is sufficient for 4 electroporations in 0.4 cm cuvettes or 10 electroporations in 0.2 cm cuvettes.

MATERIALS

Materials supplied

Ingenio Electroporation Kits are supplied in ***one*** of the following formats.

Ingenio™ Electroporation Kits			
Product No.	**Kit Components***		
	Cuvettes	**Ingenio Solution**	**Cell Droppers**
MIR 50112	25 of 0.2 cm	6.25 ml	25
MIR 50113	25 of 0.4 cm	6.25 ml	25
MIR 50115	50 of 0.2 cm	12.5 ml	50
MIR 50116	50 of 0.4 cm	12.5 ml	50
MIR 50118	100 of 0.2 cm	2 x 12.5 ml	100
MIR 50119	100 of 0.4 cm	2 x 12.5 ml	100

*Ingenio cuvettes and cell droppers are also sold separately.

Ingenio Electroporation Solution is supplied in ***one*** of the following formats.

Ingenio™ Electroporation Solution	
Product No.	**Volume**
MIR 50111	6.25 ml
MIR 50114	12.5 ml
MIR 50117	2 x 12.5 ml

Materials required, but not supplied

- Cultured cells
- Cell culture dishes
- Cell culture medium
- Electroporation instrument
- Trypsin-EDTA for dislodging adherent cells
- Purified DNA (e.g. plasmid, cosmid or linear DNA)
- Sterile tubes
- Micropipets
- Reporter or other assay as required

BEFORE YOU START:

Important Tips for Optimal Electroporations

Optimize electroporation conditions for each cell type to ensure successful results. The suggestions below generally yield highly efficient electroporations. Table 1 presents recommended starting pulse conditions for DNA electroporation into select cells.

- **DNA Purity.** Use highly purified, sterile, and contaminant-free DNA. Endotoxin-free DNA (bacterial lipopolysaccharide-free) is recommended. Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (MIR 5900) will remove endotoxin from your DNA preparation. Do not use DNA that has been purified using ethanol precipitation. Residual salt concentrations from ethanol precipitation methods can negatively affect electroporation. Do not use DNA that has been purified using miniprep kits or procedures.
- **DNA Concentration.** Use DNA stocks that range from 1 to 5 mg/ml. Use of stocks with higher concentrations may lead to non-uniform mixing with cells. Use of stocks with lower concentrations may dilute the electroporation mix.
- **Avoid storing cells in Ingenio Electroporation Solution.** Incubation of the cells in Ingenio Electroporation Solution at room temperature for more than 15 minutes may be harmful to the cells.
- **Divide cells regularly.** Maintain cells such that they are actively growing. Divide the cell culture one day before electroporation as needed. This step may not be required for slow-growing or primary cells.
- **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.
- **Post-electroporation incubation time.** Determine the optimal incubation time post-electroporation for each cell type and transfected construct. Test a range of incubation times. The optimal incubation time is generally 12-48 hours, but will vary depending on the goal of the experiment and the electroporated DNA.
- **Optimized electroporation for other cell types.** For cells other than those listed in Table 1, more optimization will be required. General pulse conditions for most cells fall within a voltage range of 200-300 V and a capacitance range of 800-1000 µF when using 0.4 cm cuvettes. For 0.2 cm cuvettes the ranges are 80-160 V and 800-1000 µF.
- **Reduce pipetting errors.** Consider making enough master mix for one or more extra electroporations to ensure consistency among similar electroporations.

PROCEDURE

The procedure below describes how to perform electroporations using the Ingenio Electroporation Solution. This protocol can be followed using any electroporator that is capable of producing exponential decay pulses.

Table 1. Recommended electroporation conditions for select cell types.

Cell Type	Cuvette Size	Cell Density (x 10^6/ml)	Voltage	Capacitance
Jurkat E6-1	0.2 cm	10	150 V	950 µF
	0.4 cm	10	260 V	950 µF
SK-N-MC	0.2 cm	5	90 V	950 µF
	0.4 cm	5	240 V	950 µF
K-562	0.2 cm	10	130 V	950 µF
	0.4 cm	10	250 V	950 µF
HL-60	0.2 cm	10	110 V	950 µF
	0.4 cm	10	275 V	950 µF
RAW 264.7	0.2 cm	5	140 V	950 µF
	0.4 cm	5	260 V	950 µF
THP-1	0.2 cm	10	125 V	950 µF
	0.4 cm	10	250 V	950 µF

All of these electroporation recommendations use an exponential decay pulse.

A. Preparation of cells 1 day before electroporation

1. Maintain cells in appropriate complete growth medium and at appropriate cell density.
2. If necessary, divide cultured cells approximately 18-24 hours prior to electroporation so that the cells reach optimal cell density at the time of electroporation. For suspension cells, optimal cell densities are approximately 1-2 million cells/ml. For adherent cells, optimal densities at electroporation are approximately 70–80% confluent. Users may also experimentally determine their own best cell densities.
3. Incubate the cells overnight.

B. Prepare for electroporation

1. Warm all required solutions to room temperature.
2. Harvest cells for electroporation. Resuspend the cells in a small volume and count, determining the number of cells per milliliter.
3. Determine the total volume of Ingenio Electroporation Solution required to perform all the desired electroporations: Multiply the number of electroporations by 0.1 ml (for 0.2 cm cuvettes) OR by 0.25 ml (for 0.4 cm cuvettes).
4. Determine the volume of cells from step B-2 required for all electroporations according to the formula:

$$\text{Cell volume required (ml)} = \frac{\text{Electroporation cell density/ml}}{\text{Harvested cell density/ml}} \text{ X Total electroporation volume (ml)}$$

5. Pipette the volume of harvested cells required into a new tube and centrifuge at 1000 x *g* for 5 minutes. Aspirate the supernatant.
6. During the centrifugation, add warm complete culture medium to a culture dish to accept cells after electroporation.
7. Resuspend the cells from step B-5 in Ingenio Electroporation Solution, using the volume determined in step B-3.

C. Perform electroporation

1. Prepare a DNA/cell mix by adding DNA to the cells in Ingenio Electroporation Solution. Use 20 µg DNA per ml of cells. Mix gently but thoroughly. Do not create bubbles. Use a unique DNA/cell mix for each different DNA construct to be electroporated.
2. Aliquot 100 µl DNA/cell mix to each 0.2 cm cuvette OR add 250 µl DNA/cell mix to each 0.4 cm cuvette.
3. Electroporate the cells at room temperature. Refer to Table 1 for appropriate pulse conditions, or determine them experimentally.
4. **Immediately** after performing each electroporation, transfer the electroporated cells into the culture vessel prepared in step B-6. For example, transfer 100 µl of electroporated cells per well of a 12-well plate. Users should determine their own best cell culture density post-electroporation depending on the cell type, expressed construct, and post-electroporation incubation period.
5. Incubate the electroporated cells in appropriate culture medium at applicable growth conditions (e.g. 37°C, 5% CO_2 in a humidified incubator) for 12-72 hours or as required. A culture medium change may be required for longer incubations.
6. Harvest cells and perform a reporter assay or other assay as required.

Do not allow cells to incubate in Ingenio Electroporation Solution for more than 15 minutes.

Transfer the cells **immediately** following electroporation to a culture vessel containing warm complete culture medium.

TROUBLESHOOTING

Problem	Solution
LOW ELECTROPORATION EFFICIENCY	
Cell concentration too low	Determine optimal electroporation cell density for each cell type.
Cells not in active growth phase	Split cells 18–24 hours before electroporation as needed.
Low-quality DNA (partially degraded or contaminated with an inhibitor, such as endotoxin)	Use highly purified, sterile, contaminant-free DNA for electroporation. Do not use DNA prepared using miniprep kits or procedures.
	Use DNA that is endotoxin-free (bacterial lipopolysaccharide-free). Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (MIR 5900) will remove endotoxin from your DNA preparation.
	Do not use DNA that has been purified using ethanol precipitation because any residual salt may negatively affect electroporation.
Post-electroporation incubation time (before assay)	Determine the optimal incubation time for each cell type and experiment.
	Test a range of incubation times (for example, from 12 to 72 hours). The optimal incubation time is generally 12-48 hours.
Cell morphology has changed	A high or low cell passage number can reduce electroporation efficiency. Use a similar passage number between experiments to ensure reproducibility.
Suboptimal DNA concentration in the electroporation mix	Use a DNA concentration of 20 µg/ml for each electroporation, or experimentally determine optimal DNA concentration for each cell type and DNA construct. Less DNA may reduce electroporation efficiency.
HIGH CELLULAR TOXICITY	
Cells not transferred immediately to culture vessel after electroporation	Transfer the cells from each cuvette to a culture dish containing warm complete culture medium immediately after each electroporation.
Electroporation pulse strength may be too high	Decrease the voltage by increments of 10 V and/or decrease the capacitance by increments of 100 µF.
Cell morphology has changed	A high or low cell passage number can reduce electroporation efficiency. Use cells at similar passage numbers between experiments to ensure reproducibility.
Endotoxin contaminated DNA	Use highly purified, sterile, contaminant-free DNA. Use cesium chloride gradient or anion exchange purified DNA. Do not use DNA prepared using miniprep kits.
	DNA contaminated with high levels of endotoxin (bacterial lipopolysaccharide) may cause high cell death depending on the cell type. Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (MIR 5900) will remove endotoxin from your DNA preparation.
DNA preparation has too much salt	High DNA salt concentration can cause changes in pulse characteristics and can result in more cell death.

RELATED PRODUCTS

Ingenio™ Cuvettes and Cell Droppers
MiraCLEAN® Endotoxin Removal Kits
Label IT® Tracker Nucleic Acid Intracellular Localization Kits
Label IT® Plasmid Delivery Controls
*Trans*IT® Transfection Reagents and Kits
*Trans*IT® *In Vivo* Gene Delivery Kits
*Trans*IT®-QR and *Trans*IT-EE Delivery Kits and Solutions

APPENDIX

Contact Mirus Bio for additional information.

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