Ingenio® Electroporation Kits and Solution

Protocol for MIR 50108, 50109, 50110, 50111, 50112, 50113, 50114, 50115, 50116, 50117, 50118, 50119

Quick Reference Protocol, SDS and Certificate of Analysis available at mirusbio.com/50111

INTRODUCTION

Ingenio® Electroporation Kits and Solution provide a universal, high efficiency, low toxicity solution for electroporation of DNA or siRNA into hard to transfect cell types. Electroporation using Ingenio® Electroporation Kits and Solution affords increased gene expression in many cell types with minimal toxicity. Ingenio® Kits and Solution are compatible with multiple conventional electroporation instruments including the Mirus Bio Ingenio® EZporator® Electroporation System, Lonza Amaxa® Nucleofector®, Bio-Rad Gene Pulser® and Harvard-BTX® electroporators. Ingenio® Kits and Solution can be used for electroporation with both exponential decay as well as square wave forms.

SPECIFICATIONS

Storage	Store the Ingenio® Electroporation Solution at 4°C. All other materials can be stored at room temperature.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.
No. of Electroporations	1 ml of Ingenio® 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 and Solution are supplied in ***one*** of the following formats:

Product No.	**Kit Components***			
	No. of Electroporations	**Ingenio® Solution**	**Cuvettes**	**Cell Droppers**
Ingenio® Electroporation Kits for Amaxa® Nucleofector®				
MIR 50112	25	1 × 6.25 ml	25 of 0.2 cm	25
MIR 50115	50	1 × 12.5 ml	50 of 0.2 cm	50
MIR 50118	100	2 × 12.5 ml	100 of 0.2 cm	100
Ingenio® Electroporation Kits for open system electroporators, e.g. Ingenio® EZporator®, Bio-Rad®, Harvard-BTX®, etc.				
MIR 50113	25	1 × 6.25 ml	25 of 0.4 cm	25
MIR 50116	50	1 × 12.5 ml	50 of 0.4 cm	50
MIR 50119	100	2 × 12.5 ml	100 of 0.4 cm	100
Ingenio® Electroporation Solution				
MIR 50111	25	1 × 6.25 ml	None*	None*
MIR 50114	50	1 × 12.5 ml	None*	None*
MIR 50117	100	2 × 12.5 ml	None*	None*

***** Ingenio® cuvettes and cell droppers are also sold separately.

For Research Use Only.

Materials required, but not supplied

- Cultured cells
- Appropriate cell culture medium
- Cell culture dish
- Purified plasmid DNA or siRNA
- Electroporation instrument
- Trypsin-EDTA for harvesting adherent cells
- Sterile tubes
- Micropipets
- Reporter assay as required

BEFORE YOU START:

Important Tips for Optimal Electroporation

Optimize electroporation conditions for each cell type to ensure successful results. The suggestions below yield high efficiency electroporation of most cell types. **Tables 1–3** on Pages 6–8 present recommended pulse conditions or program settings depending on the electroporator used.

- **Cell density and passage number**
  - **Cell division.** Approximately 18–24 hours before electroporation, passage cultures to ensure that cells are actively dividing and reach the appropriate density (i.e. 70-90% confluent for adherent cells or 2–4 $\times 10^6$ cells/ml for suspension cells) at the time of harvesting for electroporation.

    For adherent cells: Plate cells at a density of 0.8 – 3.0 x 10^5 cells/ml.

    For suspension cells: Seed cells at a density of 1 – 2 x 10^6 cells/ml.

    NOTE: This step may not be required for slow-growing or primary cells.
  - **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 $\times 10^6$ cells/ml. Higher cell densities are typically recommended for suspension cells (e.g. 10 x 10^6 cells/ml), whereas the recommended range for adherent cells is 1–5 $\times 10^6$ cells/ml.

    The final electroporation volume per cuvette is 0.1 ml and 0.25 ml for 0.2 cm and 0.4 cm cuvettes, respectively. Refer to Tables 1–3 on Pages 6–8 for starting cell densities used with Ingenio® Solution.
  - **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.**
  - **DNA.** Use highly purified, sterile, and contaminant-free DNA for electroporation. Plasmid DNA preparations that are endotoxin-free and have $A_{260/280}$ absorbance ratio of 1.8–2.0 are desirable**.**

    DNA prepared using miniprep kits is not recommended as it may contain high levels of endotoxin. We recommend using MiraCLEAN® Endotoxin Removal Kit (MIR 5900) to remove endotoxin from your DNA preparation.

    Use DNA stocks that range in concentration from 1–5 mg/ml. Use of stocks with higher concentrations may lead to non-uniform mixing with cells. Use of low concentration DNA stocks may dilute the electroporation mix and decrease electroporation efficiency.

Do not use DNA prepared using miniprep kits.

Determine the best plasmid DNA concentration for electroporation. Try DNA concentrations in the range of 5–50 µg/ml of final electroporation volume. Please refer to Tables 1–3 on Pages 6–8 for recommended DNA amounts.

- **siRNA.** Use siRNA that is highly pure, sterile, and the correct sequence.

  Determine the best siRNA concentration for electroporation. Try siRNA concentrations in the range of 250–750 nM (final concentration in cuvette).

  Mirus recommends transfecting a non-targeting or nonsense siRNA control sequence to verify that the gene expression knockdown or phenotype is attributed to the gene-specific siRNA. Additionally, targeting a gene with multiple siRNA sequences ensures that the resulting phenotype is not due to off-target effects.

- **Optimization of pulse conditions.** Ingenio® Electroporation Solution can be used with both exponential decay as well as square wave forms. To determine which pulse conditions are optimal for a certain cell type, empirical testing is required.
  - **For exponential-decay wave form**. Exponential decay pulse conditions for most cell types typically fall within a voltage range of 200–300 V and a capacitance range of 800–1050 µF when using 0.4 cm cuvettes. For 0.2 cm cuvettes, the ranges are 80–160 V and 800–1050 µF, respectively. 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) keeping the capacitance constant. If capacitance adjustment is supported by the electroporator, perform a capacitance titration by keeping the voltage constant (e.g. 220 V for 0.4 cm cuvettes; 100 V for 0.2 cm cuvettes) while varying capacitance in 100 µF increments starting at 750 µF. Refer to Table 1 on Page 6 for recommended exponential decay pulse conditions.
  - **For square wave form.** A theoretical starting point for square wave pulse conditions can typically be determined using exponential decay parameters, by halving the pulse length and increasing the voltage by ~10%, while keeping capacitance the same. For further optimization, test 10 V increments of voltage around the theoretically calculated pulse voltage. Refer to Table 2 on Page 7 for recommended square-wave pulse conditions. If exponential decay pulse conditions are not known, find the optimal square wave pulse condition by testing a range of voltages, capacitance and pulse lengths. Square wave pulse conditions for most cell types typically 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, respectively. The pulse length varies from 10–20 mSec.
  - **Amaxa® Nucleofector®.** Table 3 on Page 8 contains cell type-specific program settings for using Ingenio® Electroporation Solution with Amaxa® Nucleofector® I or II/2B electroporators. For cell types other than those listed in Table 3, we recommend following Amaxa® program guidelines as per the cell type.

- **Post-electroporation incubation time.** Determine the best incubation time post-electroporation for each cell type. For plasmid electroporation, the optimal incubation time is generally 12–48 hours but will vary depending on the goal of the experiment, the nature of the plasmid, and the half-life of the expressed protein.

  For siRNA-mediated knockdown experiments, the optimal incubation time can be determined empirically by testing a range from 24–72 hours post-electroporation, depending on the stability of the target mRNA and its encoded protein. When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hours post-electroporation is often sufficient. When quantifying knockdown efficiencies at the protein level, longer post-electroporation incubation may be necessary, particularly if the target protein has a long cellular half-life.

ELECTROPORATION PROTOCOL

The following procedure describes how to perform plasmid DNA or siRNA electroporations in 0.2 cm or 0.4 cm cuvettes using the Ingenio® Electroporation Solution and Kits. This protocol can be followed using any electroporator that is capable of producing exponential decay pulses (e.g. Mirus Bio Ingenio® EZporator® Electroporation System) or square wave pulses (e.g. Harvard Apparatus-BTX ECM 830). It can also be used with the Amaxa® Nucleofector®. **Tables 1** and **2** on Pages 6–7 present recommended pulse conditions for exponential decay and square-wave pulses, respectively. **Table 3** on Page 8 presents program settings recommended for the Amaxa® Nucleofector®.

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 x 10^5 cells/ml.

   For suspension cells: Seed cells at a density of 1–2 × 10^6 cells/ml.

2. Incubate cell cultures overnight**.**

Passage cultured cells 18–24 hours before electroporation to ensure active cell division at the time of electroporation.

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

1. Warm Ingenio® Electroporation Solution, trypsin-EDTA (if needed) and complete growth medium to room temperature.
2. Harvest cells as required per the cell type. Count cells to determine the harvested cell density/ml.
3. Determine the total electroporation volume required to perform the desired number of electroporations:

   For 0.2 cm cuvettes: Multiply the required number of electroporations by 0.1 ml.

   For 0.4 cm cuvettes: Multiply the required number of electroporations by 0.25 ml.

4. Using the harvested cell density determined in step B2, calculate the cell volume required for all electroporations according to the following formula:

$$\text{Cell volume (ml)} = \frac{\text{Final cell density/ml}}{\text{Harvested cell density/ml}} \times \text{Total electroporation volume (ml)}$$

   For suspension cells: Use a final cell density of 10 × 10^6 cells/ml.

   ***For adherent cells*:** Use a final cell density of 1–5 × 10^6 cells/ml.

5. Pipette the cell volume (from step B4) of harvested cells (from step B2) into a new tube and centrifuge at 300 × *g* for 5 minutes. Aspirate the supernatant.
6. During the centrifugation, add warm complete culture medium to a new culture dish to accept cells after electroporation. NOTE: See step C3 for plating recommendations.
7. Prepare the Ingenio® Solution/cell mixture by resuspending the cells from step 5 in the total electroporation volume (from step B3) of Ingenio® Electroporation Solution.

Warm Ingenio® Electroporation Solution to room temperature before each use.

Use only 0.2 cm cuvettes when using Amaxa Nucleofector.

8. Prepare Ingenio® Solution/nucleic acid/cell mixture by adding DNA or siRNA to the Ingenio® Solution/cell mixture. Use separate nucleic acid/cell mixtures for each different DNA or siRNA to be electroporated.

   For DNA electroporation: Use 20 µg DNA per ml of cells as a starting point. For further optimization, please refer to Before You Start on Page 2.

   For siRNA electroporation: Use 250 nM siRNA (final concentration in cuvette) as a starting point. For further optimization, please refer to Before You Start on Page 3.

   Mix gently but thoroughly. Do not create bubbles.

To ensure consistency among similar electroporations, prepare a nucleic acid/cell mixture master mix for each plasmid or siRNA being electroporated.

To reduce pipetting errors, prepare enough master mix for one or more extra electroporations.

C. Perform electroporation

1. Aliquot Ingenio® Solution/nucleic acid/cell mixture into different cuvettes for each electroporation.

   For 0.2 cm cuvettes: Pipet 100 µl Ingenio® Solution/nucleic acid/cell mixture to each 0.2 cm cuvette.

   For 0.4 cm cuvettes: Pipet 250 µl Ingenio® Solution/nucleic acid/cell mixture to each 0.4 cm cuvette.

2. Electroporate the cells at room temperature. The pulse conditions or program settings required for electroporation will vary depending on the cell type and the electroporator used and need to be determined experimentally.

   For exponential decay electroporators: **Table 1** on Page 6 presents recommended pulse conditions including voltage and capacitance used for exponential decay pulses. For cells other than those listed in Table 1, further optimization will be required.

   General exponential decay pulse conditions for most cell types fall within a voltage range of 200–300 V and a capacitance range of 800–1050 µF when using 0.4 cm cuvettes. For 0.2 cm cuvettes, the ranges are 80–160 V and 800–1050 µF, respectively.

   For square wave electroporators: **Table 2** on Page 7 presents recommended pulse conditions including voltage, capacitance and pulse length for square-wave pulses. For cells other than those listed in Table 2, further optimization will be required.

   General square wave pulse conditions for most cell types 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, respectively. The pulse length varies from 10–20 mSec.

   For Amaxa® Nucleofector®: **Table 3** on Page 8 presents program settings recommended for Amaxa® Nucleofector®. For program settings for cells other than those listed in Table 3, Mirus recommends following Amaxa guidelines as per the cell type.

   In addition to choices such as voltage and capacitance settings, some electroporators also offer a choice of resistance (ohms, Ω). Using Ingenio® Electroporation Solution, the ideal resistance setting is: none, zero ohms (Ω) or ∞. If your electroporator requires a resistance setting other than zero, use the lowest possible resistance.

3. Transfer the electroporated cells into the culture dish (from step B6), e.g. 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, nucleic acid electroporated**,** and post-electroporation incubation period.

Do not allow cells to incubate in Ingenio® Electroporation Solution for more than 30 minutes post electroporation.

4. Incubate the electroporated cells in appropriate culture medium for 12-72 hours or as required. A culture medium change may be required for longer incubations.

5. Harvest cells and assay as required.

Table 1. Recommended program settings for electroporation using Ingenio® Electroporation Solution and Kits with exponential decay pulse electroporators, e.g.Mirus Bio Ingenio® EZporator® Electroporation System, Bio-Rad Gene Pulser® XCell™ or Harvard Apparatus BTX® ECM® 630 electroporators.

Cell Type	Cuvette Size (cm)	Cell Density ($x10^6$) cells/ml	DNA (µg)	Electroporation Volume (µl)	Voltage (V)	Capacitance (µF)
Primary Human Keratinocyte	0.2	2	2	100	150	950 - 1050
	0.4		5	250	220	950 - 1050
Primary MEFs	0.2	5	2	100	150	950 - 1050
	0.4		5	250	230	950 - 1050
Primary Rat Cortical Neuron	0.2	1	2	100	120	950 - 1050
	0.4		–	–	–	–
A-549	0.2	5	–	–	–	–
	0.4		5	250	280	950 - 1050
BHK-21	0.2	10	2	100	150	950 - 1050
	0.4		5	250	280	950 - 1050
CHO-K1	0.2	5	2	100	150	950 - 1050
	0.4		5	250	280	900 - 1050
COS-7	0.2	5	2	100	150	950 - 1050
	0.4		5	250	260	950 - 1050
HEK-293	0.2	5	2	100	160	950 - 1050
	0.4		5	250	250	950 - 1050
HEK-293T	0.2	5	–	–	–	–
	0.4		5	250	250	950 - 1050
HeLa	0.2	3	2	100	130	950 - 1050
	0.4		5	250	260	950 - 1050
Hepa	0.2	5	2	100	160	950 - 1050
	0.4		–	–	–	–
HepG2	0.2	5	2	100	170	950 - 1050
	0.4		5	250	250	950 - 1050
HL-60	0.2	10	2	100	150	950 - 1050
	0.4		5	250	275	950 - 1050
HUV-EC	0.2	3	–	–	–	–
	0.4		5	250	250	950 - 1050
Jurkat E6-1	0.2	10	2	100	150	950 - 1050
	0.4		5	250	260	950 - 1050
K562	0.2	10	2	100	130	950 - 1050
	0.4		5	250	250	950 - 1050
MCF-7	0.2	3	2	100	150	950 - 1050
	0.4		–	–	–	–
NIH-3T3	0.2	10	2	100	160	950 - 1050
	0.4		5	250	260	950 - 1050

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Table 1: Exponential Decay Pulse Conditions, continued.

Cell Type	Cuvette Size (cm)	Cell Density ($x10^6$) cells/ml	DNA (µg)	Electroporation Volume (µl)	Voltage (V)	Capacitance (µF)
NIKS	0.2	2	2	100	170	950 - 1050
	0.4		5	250	280	950 - 1050
PC-12	0.2	3	2	100	130	950 - 1050
	0.4		5	250	240	950 - 1050
RAW 264.7	0.2	5	2	100	150	950 - 1050
	0.4		5	250	260	950 - 1050
SH-SY5Y	0.2	5	–	–	–	–
	0.4		5	250	250	950 - 1050
SK-BR-3	0.2	5	2	100	160	950 - 1050
	0.4		5	250	260	950 - 1050
SK-N-MC	0.2	5	2	100	90	950 - 1050
	0.4		5	250	240	950 - 1050
THP-1	0.2	10	2	100	140	950 - 1050
	0.4		5	250	250	950 - 1050
U-937	0.2	10	–	–	–	–
	0.4		5	250	260	950 - 1050
Vero	0.2	5	2	100	170	950 - 1050
	0.4		–	–	–	–
Other cell types	0.2	5–10	2	100	80–160	800–1050
	0.4		5	250	200–300	800–1050

Table 2. Recommended program settings for electroporation using Ingenio® Electroporation Solution and Kits with square-wave electroporators, e.g. Bio-Rad Gene Pulser XCell or Harvard Apparatus-BTX ECM 830 electroporators.

Cell Type	Cuvette Size (cm)	Cell Density ($x10^6$) cells/ml	DNA (µg)	Electroporation Volume (µl)	Voltage (V)	Capacitance (µF)	Pulse Length (mSec)
Primary Human Keratinocyte	0.2	2	2	100	170	950	10
	0.4		5	250	250	950	15
Primary MEFs	0.2	5	2	100	170	950	10
	0.4		5	250	280	950	15
A-549	0.2	5	–	–	–	–	–
	0.4		5	250	280	950	15
Jurkat E6-1	0.2	10	2	100	180	950	10
	0.4		5	250	275	950	15
NIH-3T3	0.2	10	2	100	160	950	10
	0.4		5	250	260	950	15
NIKS	0.2	2	2	100	180	950	10
	0.4		–	–	–	–	–
Other cell types	0.2	5–10	2	100	80–160	800–1000	10–20
	0.4		5	250	200–300	800–1000	10–20

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Table 3. Recommended program settings for electroporation using Ingenio® Electroporation Solution and Kits with Amaxa® Nucleofector® (100 µl electroporation volume in 0.2 cm cuvettes).

Cell Type	Program Setting	DNA (µg)	Cell Density ($x10^6$) cells/ml
Primary Human Keratinocyte	T-018	2	2
Primary MEFs	A-023, T-020	2	5
Primary Rat Cortical Neuron	O-003	2	1
A-549	X-001	2	2
BHK-21	A-031	2	10
CHO-K1	U-023	2	5
COS-7	W-001	2	5
HEK-293	Q-001	2	5
HEK-293T	Q-001	2	5
HeLa	I-013	2	3
Hepa	T-028	2	5
HepG2	T-028	2	5
HL-60	T-019	2	10
HUV-EC	V-001	2	3
Jurkat E6-1	X-001	2	10
K562	T-016	2	10
MCF-7	P-020	2	3
NIH-3T3	U-030	2	10
NIKS	T-018	2	2
PC-12	U-029	2	3
RAW 264.7	D-032	2	5
SK-BR-3	E-009	2	5
SK-N-MC	S-020	2	5
THP-1	V-001	2	10
U-937	W-001	2	10
Vero	V-001	2	5
Other cell types	Follow Amaxa® Nucleofector® recommendations as per the cell type		

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TROUBLESHOOTING GUIDE

Problem	Solution
LOW ELECTROPORATION EFFICIENCY	
Cell density not optimal at time of electroporation	Determine the best cell density for each cell type to maximize electroporation efficiency. Use this cell density in subsequent experiments to ensure reproducibility. For most suspension cells, a cell density of 10×10^6 cells/ml is recommended at 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 subtype.
Cells not in actively dividing at the time of electroporation	Passage the cells at least 18–24 hours before electroporation to ensure that the cells are actively dividing and reach optimal cell density at 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 5–50µg/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.
	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.
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, use Ingenio® Electroporation Solution to deliver a positive control such as a luciferase, beta-galactosidase 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** Mirus Bio prelabeled *Label* IT Plasmid Delivery Controls (please refer to Related Products on Page 11).
Suboptimal siRNA concentration	The optimal siRNA concentration generally ranges between 250–750 nM final concentration (in cuvette). 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 on-target effects.
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, use Ingenio® Electroporation Solution to deliver a siRNA targeted against a ubiquitous gene, e.g. GAPDH or Lamin A/C, followed by target western blotting or mRNA quantification.
	To assess delivery efficiency of siRNA, use Mirus Bio *Label* IT siRNA Tracker™ Intracellular Localization Kits or a prelabeled *Label* IT RNAi Delivery Control (please refer to Related Products on Page 11).

TROUBLESHOOTING GUIDE, continued

Problem	Solution
LOW ELECTROPORATION EFFICIENCY	
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.
HIGH CELLULAR TOXICITY	
Electroporation pulse strength might be too high	Decrease voltage in 10 V increments. If capacitance settings can be adjusted on the electroporation instrument, decrease capacitance in 100 µF increments.
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 the DNA has been 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 electroporation mix	Reduce the amount of DNA used for electroporation. DNA concentrations as low as 5ug/ml of final electroporation volume can be used.
	Compare toxicity levels against a cells + Ingenio® Solution control to assess the effects of the DNA transfected. If you still see toxicity, it is likely due to the Ingenio®/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.

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RELATED PRODUCTS

- Ingenio® EZporator® Electroporation System
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® RNAi Delivery Controls
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits
- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT-PRO® Transfection Reagent and Kit
- *Trans*IT-TKO® Transfection Reagent
- *Trans*IT-siQUEST® Transfection Reagent
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits
- *Trans*IT®-QR and *Trans*IT®-EE Delivery Solutions and Kits

For details on our products, visit www.mirusbio.com

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at: mirusbio.com/ra