Ingenio® Electroporation Kits and Solutions

Quick Reference Protocol

Instructions for use with MIR 50108, 50109, 50110, 50111, 50112, 50113, 50114, 50115, 50116, 50117, 50118, 50119

Full protocol, SDS and Certificate of Analysis available mirusbio.com/50111

SPECIFICATIONS

Storage	Store Ingenio® Electroporation Solution at 4°C.
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 and 10 electroporations in 0.2 cm cuvettes.

INGENIO® ELECTROPORATION PROTOCOL

Full protocol and additional documentation available at ***mirusbio.com/50111***

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 x 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 needed) and complete growth medium to room temperature.
2. Harvest cells as required per cell type. Count cells to determine 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 adherent cells: Use a final cell density of 1 - 5 x 10^6 cells/ml

   For suspension cells: Use a final cell density of 10 x 10^6 cells/ml.
5. Pipette the cell volume (from B4) of harvested cells into a new tube and centrifuge at 300 *x 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 (see C3 for plating recommendations).
7. Prepare the Ingenio® Solution/cell mixture by resuspending cells from step B5 in the electroporation volume (calculated in B3) of Ingenio® Electroporation Solution.
8. Prepare Ingenio Solution/nucleic acid/cell mixture by adding DNA or siRNA to Ingenio® Solution/cell mixture. Use separate nucleic acid/cell mixture for each different DNA or siRNA to be electroporated. Mix gently but thoroughly. Do not create air bubbles.

   For DNA electroporation: Use 20 µg DNA per 1 ml of cells as a starting point.

   For siRNA electroporation: Use 250 nM siRNA (final concentration) as a starting point.

   NOTE: For further optimization, refer to the Ingenio® Full Protocol.

INGENIO® ELECTROPORATION PROTOCOL

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C. Perform Electroporation

1. Aliquot Ingenio® Solution/nucleic acid/cell mixture into cuvettes for electroporation.
   For 0.2 cm cuvettes: Pipet 100 µl Ingenio®/nucleic acid/cell mixture per cuvette
   For 0.4 cm cuvettes: Pipet 250 µl Ingenio®/nucleic acid/cell 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. Refer to the Tables 1 - 3 on pages 3 and 4 for recommended pulse conditions based on cell type. If your cell type is not listed, the correct settings should be determined experimentally.
   For exponential decay electroporators: Refer to Table 1 on page 3 for cell type-specific recommendations for exponential decay pulses. For most cell types, optimal conditions fall within the following ranges:
   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)
   For square wave electroporators: Refer to Table 2 on Page 4 for cell type-specific recommendations for square wave pulses. For most cell types, optimal conditions fall within the following ranges:
   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)
   NOTE: The pulse length varies from 10-20 mSec.
   For Amaxa Nucleofector: Refer to Table 3 on Page 4 for cell type-specific program settings for Amaxa™ Nucleofector™ I, II /2B electroporators. For cell types other than those listed in Table 3, Mirus recommends following Amaxa™ program guidelines as per the cell type.
3. Transfer the electroporated cells into the prepared culture dish (step B6).
   Example: Transfer 100 µl of electroporated cells per well of a 12-well plate.
   NOTE: Users should determine the optimal post-electroporation cell culture density depending on the cell type, 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.

Reagent Agent®

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INGENIO® ELECTROPORATION PROGRAM SETTING RECOMMENDATIONS

Table 1: Recommended program settings for electroporation using Ingenio® Electroporation Solution with exponential decay pulse electroporators, e.g. 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
	0.4		5	250	220	950
Primary MEFs	0.2	5	2	100	150	950
	0.4		5	250	230	950
Primary Rat Cortical Neuron	0.2	1	2	100	120	950
	0.4		–	–	–	–
A-549	0.2	5	–	–	–	–
	0.4		5	250	280	950
BHK-21	0.2	10	2	100	150	950
	0.4		5	250	280	950
CHO-K1	0.2	5	2	100	150	950
	0.4		5	250	280	900
COS-7	0.2	5	2	100	150	950
	0.4		5	250	260	950
HEK 293, HEK 293T	0.2	5	2	100	160	950
	0.4		5	250	250	950
HeLa	0.2	3	2	100	130	950
	0.4		5	250	260	950
Hepa	0.2	5	2	100	160	950
	0.4		–	–	–	–
HepG2	0.2	5	2	100	170	950
	0.4		5	250	250	950
HL-60	0.2	10	2	100	150	950
	0.4		5	250	275	950
HUV-EC	0.2	3	–	–	–	–
	0.4		5	250	250	950
Jurkat E6-1	0.2	10	2	100	150	950
	0.4		5	250	260	950
K562	0.2	10	2	100	130	950
	0.4		5	250	250	950
MCF-7	0.2	3	2	100	150	950
	0.4		–	–	–	–
NIH-3T3	0.2	10	2	100	160	950
	0.4		5	250	260	950
NIKS	0.2	2	2	100	170	950
	0.4		5	250	280	950
PC-12	0.2	3	2	100	130	950
	0.4		5	250	240	950
RAW 264.7	0.2	5	2	100	150	950
	0.4		5	250	260	950
SH-SY5Y	0.2	5	–	–	–	–
	0.4		5	250	250	950
SK-BR-3	0.2	5	2	100	160	950
	0.4		5	250	260	950
SK-N-MC	0.2	5	2	100	90	950
	0.4		5	250	240	950
THP-1	0.2	10	2	100	140	950
	0.4		5	250	250	950
U-937	0.2	10	–	–	–	–
	0.4		5	250	260	950
Vero	0.2	5	2	100	170	950
	0.4		–	–	–	–
Other cell types	0.2	5–10	2	100	80–160	800–1000
	0.4		5	250	200–300	800–1000

INGENIO® ELECTROPORATION PROGRAM SETTING RECOMMENDATIONS

Table 2. Recommended program settings for electroporation using Ingenio® Electroporation Solution 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

Table 3. Recommended program settings for electroporation using Ingenio® Electroporation Solution with Amaxa™ Nucleofector™ I and II/2B instruments (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, HEK 293T	Q-001	2	5
HeLa	I-013	2	3
Hepa, 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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Rev.A 010918