

Entranster™ –E Electroporation Solution

Cat. No. 98668

For Research Only

Description

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

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

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** present recommended pulse conditions or program settings depending on the electroporator used.

• Cell density and passage number

- **Cell division.** Plate the cells 18–24 hours before electroporation at a density of $1\text{--}2 \times 10^6$ cells/ml to ensure that the cells are actively dividing and reach the appropriate cell density (generally $2\text{--}4 \times 10^6$ cells/ml) at the time of harvesting for electroporation. 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 final cell density for electroporation falls in the range of $1\text{--}10 \times 10^6$ cells/ml of the final electroporation volume. For suspension cells, it is best to use higher cell densities closer to 10×10^6 cells/ml. For adherent cells, a range of $1\text{--}5 \times 10^6$ cells/ml is recommended. The final electroporation volume per cuvette is 0.1 ml and 0.25 ml when using a 0.2 cm and 0.4 cm cuvette, respectively. Please refer to Tables 1–3 for starting cell densities used with Entranster™ –E.

- **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 A260/280 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.

Do not use DNA that has been purified using only ethanol precipitation. Residual salt concentrations from ethanol precipitation methods can cause arcing and negatively affect electroporation.

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 stocks with lower concentrations may dilute the electroporation mix.

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 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. Engreen 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.** Entranster™ –E Electroporation Solution can be used with both exponential decay as well as square wave forms. Certain cell types transfect better with square wave pulses while others respond better to exponential decay. Therefore, empirical testing is required for each cell type.

- **For exponential-decay wave form.** General exponential decay 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. For electroporations using 0.4 cm cuvettes, test a voltage range of 200–300 V and a capacitance range of 800–1000 µF. First, keep the voltage constant at 220 V while varying capacitance in 100 µF increments starting at 750 µF. Following this, vary voltage in 10 V increments starting at 200 V keeping capacitance constant at the value determined from the capacitance titration. Please refer to Table 1 for recommended exponential decay pulse conditions.

- **For square wave form.** Generally, a theoretical starting point for square wave pulse conditions can 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. Please refer to Table 2 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. 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.

- Amaxa Nucleofector. When using Entranster™-E Electroporation Solution with Amaxa Nucleofector, use the program setting recommended for your target cell type by Amaxa. Please refer to Table 3 for recommended program settings with Amaxa Nucleofector.
- **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 Entranster™-E Electroporation Solution and Kits. This protocol can be followed using any electroporator that is capable of producing exponential decay or square wave pulses. It can also be used with the Amaxa Nucleofector. **Tables 1 and 2** present recommended pulse conditions for exponential decay and square-wave pulses, respectively. **Table 3** 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 so that they reach optimal cell density at the time of electroporation.

For adherent cells: Plate cells at 70–80% confluence.

For suspension cells: Plate cells at a density of $1-2 \times 10^6$ cells/ml.

2. Incubate cell cultures overnight.

B. Prepare Entranster™-E Solution/nucleic acid/ cell mixture for electroporation (Immediately before electroporation)

- 1 Warm Entranster™-E Electroporation Solution, trypsin-EDTA and complete growth medium to room temperature.
- 2 Trypsinize and harvest adherent cells for electroporation. Trypsinization is not required for suspension cells. Resuspend the cells in a small volume of growth medium and count, determining 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 formula:

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

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

* *For adherent cells:* Use a final cell density of $1-5 \times 10^6$ cells/ml.

5 Pipette the cell volume (from step B4) of harvested cells (from step B2) into a new tube and centrifuge at $1000 \times g$ 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.

7 Prepare the Entranster™-E Solution/cell mixture by resuspending the cells from step 5 in the total electroporation volume (from step B3) of Entranster™-E Electroporation Solution.

8. Prepare the Entranster™-E Solution/nucleic acid/cell mixture by adding DNA or siRNA to the Entranster™-E 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 2.

For siRNA electroporation: Use 250 nM siRNA (final concentration) as a starting point. For further optimization, please refer to Before you Start 3. Mix gently but thoroughly. Do not create bubbles.

C. Perform electroporation

1. Aliquot Entranster™-E Solution/nucleic acid/cell mixture into different cuvettes for each electroporation.

For 0.2 cm cuvettes: Pipet 100 µl Entranster™-E Solution/nucleic acid/cell mixture to each 0.2 cm cuvette.

For 0.4 cm cuvettes: Pipet 250 µl Entranster™-E 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 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 800–1000 µF when using 0.4 cm cuvettes. For 0.2 cm cuvettes, the ranges are 80–160 V and 800–1000 µF, respectively.

For square wave electroporators: Table 2 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 presents program settings recommended for Amaxa Nucleofector. For program settings for cells other than those listed in Table. Engreen

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 Entranster™-E 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 μ l 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.

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 Entranster™-E 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 ($\times 10^6$) cells/ml	DNA (μ g)	Electroporation Volume (μ l)	Voltage (V)	Capacitance (μ F)
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	0.2	5	2	100	160	950
	0.4		5	250	250	950
HEK-293T	0.2	5	-	-	-	-
	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

Table 1: Exponential Decay Pulse Conditions. Continued.

Cell Type	Cuvette Size (cm)	Cell Density ($\times 10^6$) cells/ml	DNA (μg)	Electroporation Volume (μl)	Voltage (V)	Capacitance (μF)
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	900
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

Table 2. Recommended program settings for electroporation using Entranster™-E 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 ($\times 10^6$) 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	800-100	10-20

Table 3. Recommended program settings for electroporation using Entranster™-E Electroporation Solution with Amaxa Nucleofector (100 μl electroporation volume in 0.2 cm cuvettes).

Cell Type	Program Setting	DNA (μg)	Cell Density ($\times 10^6$) 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

Table 3: Recommended program settings for electroporation using Entranster™-E Electroporation Solution with Amaxa Nucleofector (100 µl electroporation volume in 0.2 cm cuvettes). Continued.

Cell Type	Program Setting	DNA (µg)	Cell Density (x10 ⁶) cells/ml
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		

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 ×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 A260/280 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. 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. Do not use DNA purified using ethanol precipitation. High residual salt from ethanol precipitation can cause racing and will be detrimental to electroporation.
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of the plasmid DNA.
Proper experimental controls plasmid were not included for plasmid delivery	To verify efficient electroporation, use Entranster™-E Electroporation Solution to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid.
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 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	1.Cells alone 2.Serum-free medium + Entranster™-E Electroporation Solution + a non-targeting siRNA To verify efficient transfection and knockdown, use Entranster™-E 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.
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 the voltage by increments of 10 V and/or decrease the capacitance by increments of 100 µF
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. 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 5µg/ml of final electroporation volume can be used. Compare toxicity levels against a cells + Entranster™-E Solution control to assess the effects of the DNA transfected. If you still see toxicity, it is likely due to the Entranster™-E/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.

Storage and Stability

- Entranster™-E Electroporation Solution is shipped at +4°C.
- Entranster™-E Electroporation Solution is stabilized for extended storage at +4°C through the expiration date printed on the label (one year from the date of manufacture) when very tightly closed.

Related Products

Entranster™-H4000: Transfect DNA into cells.

Entranster™-D4000: Transfect DNA into cells.

Entranster™-R4000: Transfect siRNA into animal cells.

Notice to Purchaser

Purchaser represents and warrants that Entranster™-E Electroporation Solution will be used only for research purposes. Transfected cells, materials produced, and any data derived from the use of Entranster™-E Electroporation Solution, may be used only for the internal research of Purchaser whether Purchaser is a “for-profit” or a “non-profit” organization. Under no circumstances may Entranster™-E Electroporation Solution be used by Purchaser or any third party for a commercial purpose unless Purchaser has negotiated a license for commercial use with Engreen Biosystem (contact information: License@Engreen.com.cn). For purposes of the foregoing sentence, “commercial purpose” shall mean use of Entranster™-E Electroporation Solution for profit or commercial gain. By using Entranster™-E Electroporation Solution, Purchaser agrees to be bound by the above terms. If Purchaser wishes not to be bound by these terms, Purchaser agrees to return the Entranster™-E Electroporation Solution to Engreen Biosystem for a full refund.

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