Gene Pulser® Electroporation Buffer Reagent

Catalog #	Volume
165-2676	10 x 1.8 ml
165-2677	30.0 ml

For research only. Store at 4°C.

Storage and Stability

Gene Pulser electroporation buffer reagent is shipped on ice. Store at 4°C upon receipt. Gene Pulser electroporation buffer is stable for 6 months from date of purchase when stored at 4°C.

Handling Precautions

Gene Pulser electroporation buffer reagent is intended for the delivery of siRNAs and plasmid DNA into cultured mammalian cells. It is nuclease-free to prevent the degradation of siRNAs or plasmid DNA. Use gloves and nuclease-free reagents, pipets, and tubes, as well as good tissue culture practices.

Contents

Electroporation buffer contains 10 x 1.8 ml (catalog #165-2676) or 30.0 ml (catalog #165-2677) of a proprietary buffer formulation. 1 ml is generally sufficient for 5–10 transfections in 0.2 cm cuvettes, 1–2 transfections in 0.4 cm cuvettes, or 5–10 transfections in a 96-well plate.

Note: One 30 ml bottle is sufficient for electroporating two entire plates (96-, 24-, or 12-well plates). You do not have to fill all wells in each plate

Overview

Electroporation is the preferred method for introducing biomolecules into cells, especially into cells that are resistant to other methods of transfection. However, this method requires an effective electroporation buffer and optimized conditions to ensure high cell viability after electroporation.

Gene Pulser electroporation buffer reagent was developed for the transfection of siRNAs and plasmid DNA into a variety of cell types, resulting in high efficiency and low cytotoxicity.

Gene Pulser electroporation buffer reagent allows transfection using a variety of protocols and conditions, can be used with any multiwell plate format or cuvette size, and can be used in Gene Pulser® II, Gene Pulser Xcell™, Gene Pulser MXcell™, and other electroporation systems.

For best results, it is important to determine the optimal amount of siRNA or plasmid DNA, and the optimal electroporation conditions for any given cell line.

Recommendations for Optimal Results

Gene Pulser electroporation buffer reagent has been developed to achieve consistent transfection efficiencies using a broad range of cell types with an easy-to-use protocol.

Optimum transfection efficiencies are achieved by adjusting:

- siRNA or plasmid DNA concentration
- Cell density at the time of transfection
- Waveform (exponential decay or square waveforms)
- Voltage
- Pulse length

Once maximum transfection efficiency has been established, the conditions should be kept constant between experiments for any particular cell line.

Optimization

Determining the optimum electroporation conditions is essential to maximize the transfection efficiency of siRNAs, to obtain the best gene silencing results, and to minimize cellular toxicity. The same is true for the delivery of plasmid DNA.

Optimize parameters for every cell line using the guidelines in Table 1.

Table 1. Electroporation optimization guidelines.

Parameter		Optimization range
Voltage	Square waveforms	50–400 V (in 10 V increments)
	Exponential waveforms	100–350 V (in 50 V increments)
Pulse length (for square waveforms)		10–25 ms
Capacitance (for exponential waveforms)		500–1000 µF
Vessel size*		0.4 cm^2 cuvette** or 96-, 24-, or 12-well electroporation plate
Cell density	Adherent cells	1–5 x 10^6 cells/ml
	Suspension cells	2–10 x10^6 cells/ml
siRNA concentration		10–100 nM

* See Table 2 for maximum values.

** If using a 0.2 cm cuvette, reduce the voltage by 50%.

Transfection Protocol

1. Harvest and count the cells.
   a. The cells should be passaged the day before electroporation. All cell types should be harvested when they are actively growing. If working with adherent cells, trypsinize the cells to detach them, add growth medium, and then pellet the cells. If working with suspension cells, pellet the cells.
   b. After pelleting the cells, remove the medium and wash the cells once with PBS, by carefully pipetting them. Take an aliquot and count the cells.
2. Prepare the cells for electroporation.
   a. Aliquot the number of cells needed to perform the experiment. For adherent cells, we recommend using 1 x 10^6 cells/ml, but we have successfully used 0.5–5 x 10^6 cells/ml. For suspension cells, we recommend using 2–3 x 10^6 cells/ml.
   b. Pellet the cells.
   c. Aspirate the PBS and resuspend the cells in the appropriate volume of electroporation buffer reagent (1 ml per 1 x 10^6 of adherent cells, and 1 ml per 2–3 x 10^6 of suspension cells).
   d. Add nucleic acid. For siRNA electroporation, use 10–100 nM of siRNA. For plasmid DNA electroporation, use 5–20 µg/ml.
3. Electroporate cells.
   a. Choose the proper vessel for your sample size (see Table 2).

Table 2. Proper vessel based on sample size.

Sample Volume	Vessel
100–200 µl	0.2 cm cuvette
400–800 µl	0.4 cm cuvette
100–200 µl	96-well plate
500–800 µl	24-well plate
1.0–1.5 ml	12-well plate

   b. Electroporate the sample using optimized conditions.
   c. Transfer cells to tissue culture dishes containing growth media.
   d. Incubate cells at 37°C in a humidified CO_2 incubator until ready to be assayed. After 24 hr, change the growth medium.
4. Assess transfection efficiency.
   a. Fluorescently labeled siRNAs can be used to determine the transfection efficiency for siRNA delivery. Transfection efficiency can be measured by fluorescent microscopy or flow cytometry.
   b. For plasmid delivery, the transfection efficiency can be determined by electroporating plasmids expressing reporter genes such as GFP or β-galactosidase.