



# Gene Pulser® Electroprotocols

\* We recommend adapting this protocol to use the Gene Pulser electroporation buffer (catalog #165-2676, 165-2677), which increases cell viability and transfection efficiency in mammalian cell lines.

**Cell Type** Mammalian, adherent  
**Species Used** Human, HeLa, epithelial carcinoma

**Molecules Electroporated** DNA: supercoiled DNA used for transient transfections; linearized DNA used for stable transfections.

## Before the Pulse

<b>Cell growth medium</b>	DMEM, 10% Fetal Calf Serum [FCS] (GIBCO/BRL, Sigma)	<b>Growth phase at harvest</b>	50 to 70% confluency
<b>Wash solution</b>	Wash two times in electroporation buffer	<b>Pre-pulse incubation</b>	4° C, 10 min.

## The Pulse

<b>Electroporation Temperature</b>	Room temperature	<b>Instruments Used</b>	Gene Pulser® apparatus & Capacitance Extender
<b>Electroporation Medium*</b>	HEPES Buffered Saline, 6mM glucose	<b>Cuvette Gap</b>	0.4 cm
<b>Cell Density</b>	5 x 10(6) cells/pulse for transient assay; 5 x 10(5) cells/pulse, stable transfection	<b>Voltage</b>	0.170 kV
<b>Volume of Cells</b>	0.5 ml	<b>Field Strength</b>	0.425 kV/cm
<b>DNA Concentration</b>	10 µg / pulse	<b>Capacitor</b>	960 µF
<b>DNA Resuspension Buffer</b>	Not given; pulse volume: 0.8 ml	<b>Resistor</b>	(Pulse Controller) Ω none
<b>Volume of DNA</b>	Not given; pulse volume: 0.8 ml	<b>Time Constant</b>	30 msec

## After the Pulse

<b>Outgrowth Medium</b>	DMEM, 10% Fetal Calf Serum (FCS)	<b>Relevant Publications and/or Comments</b>	<b>Note:</b> exponential values designated in parentheses.
<b>Outgrowth Temperature</b>	37 °C	<b>Note:</b>	Stable transfections generally do not use carrier DNA. Also, the level of selective agent required to kill off non-transfected cells needs to be established before transfection. The level required should kill non-transfected cells in approximately 7 days.
<b>Length of Incubation</b>	48 to 72 hrs.		
<b>Selection Method or Assay Used</b>	G418 (stable transfections) and transient assays		
<b>Electroporation Efficiency</b>	Not given	<b>HBS:</b>	10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl <sub>2</sub>
<b>Per Cent Survival</b>	about 50 %		

**Name of Submitter**  
**Institution Address**

**Telephone Number**  
**Fax Number**  
**Date Submitted** 7/1/90  
**Survey Number** 097  
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