



# 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.

<b>Cell Type</b>	Mammalian, suspension	<b>Molecules Electroporated</b>	DNA: linearized DNA used for stable transfections.
<b>Species Used</b>	Mouse, X-63, myeloma [p3 X63 - Ag8.653]		

## 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>	Phosphate Buffered Saline	<b>Cuvette Gap</b>	0.4 cm
<b>Cell Density</b>	5 x 10 (5) cells/pulse, stable transfection	<b>Voltage</b>	0.180 kV
<b>Volume of Cells</b>	0.5 ml	<b>Field Strength</b>	0.45 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>	24 msec

## After the Pulse

<b>Outgrowth Medium</b>	DMEM, 10% Fetal Calf Serum (FCS)	<b>Relevant Publications and/or Comments</b>
<b>Outgrowth Temperature</b>	37 °C	<b>Note:</b> exponential values designated in parentheses.
<b>Length of Incubation</b>	48 to 72 hrs.	<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>Selection Method or Assay Used</b>	G418 (stable transfections)	<b>PBS:</b> 1x = 8g NaCl, 0.2g KCl, 0.2g KH <sub>2</sub> PO <sub>4</sub> , 1.15g Na <sub>2</sub> HPO <sub>4</sub>
<b>Electroporation Efficiency</b>	Not given	
<b>Per Cent Survival</b>	about 50 %	

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

**Telephone Number**  
**Fax Number**  
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