



# 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</b>	Proteins
<b>Species Used</b>	Monkey, CV-1, kidney; Mouse, 3T3, embryo; Mouse, p3x63AG8, myeloma; Human: HeLa, epithelial carcinoma	<b>Electroporated</b>	

## Before the Pulse

<b>Cell growth medium</b>	DMEM (GIBCO/BRL, Sigma)	<b>Growth phase at harvest</b>	50 to 60% confluent cells
<b>Wash solution</b>	(See notes)	<b>Pre-pulse incubation</b>	5 minutes

## The Pulse

<b>Electroporation Temperature</b>	0 °C (See notes)	<b>Instruments Used</b>	Gene Pulser® apparatus
<b>Electroporation Medium*</b>		<b>Cuvette Gap</b>	0.4 cm
<b>Cell Density</b>	5 x 10 <sup>(6)</sup> cells / ml	<b>Voltage</b>	0.5 to 0.75 kV
<b>Volume of Cells</b>	0.2 µl	<b>Field Strength</b>	1.25 to 1.875 kV/cm
<b>DNA Concentration</b>	Not given	<b>Capacitor</b>	25 µF
<b>DNA Resuspension Buffer</b>	Not given	<b>Resistor</b>	(Pulse Controller) Ω none
<b>Volume of DNA</b>	Not given	<b>Time Constant</b>	2 to 4 msec

## After the Pulse

<b>Outgrowth Medium</b>	Pore-Resealing Buffer (see notes) for 10 min. ,37° C; follow with DMEM plus 10% Fetal Calf Serum.	<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>	Not given	<b>Ref:</b> M.R. Mitchell et. al. (1988) <i>Experientia</i> <b>44</b> : 199-203.
<b>Selection Method or Assay Used</b>	Not given	M.R. Michel et. al. (1990) <i>J.Virol.</i> <b>64</b> : 5123-5131.
<b>Electroporation Efficiency</b>	Not given	Elgizoli, M. et. al. (1989) <i>J. Virol.</i> <b>63</b> : 2921-2928.
<b>Per Cent Survival</b>	Not given	<b>Electroporation Buffer:</b> 20 mM PIPES, pH 7; 128 mM K-glutamate, 5mM ATP, 5 mM GTP, 10 µm Ca-Acetate, 2 mM Mg-Acetate & amino acid concentration corresponding to that of DMEM media.
		<b>Pore-Resealing Buffer:</b> 20 mM PIPES, pH 7.0, 128 mM K-glutamate,10 µm Ca-Acetate, 2 mM Mg-Acetate

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