



# 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: EBV based vectors, 12-20 kB, closed circular
<b>Species Used</b>	Human, UC729-6, lymphoblastoid, B-cells		

## Before the Pulse

<b>Cell growth medium</b>	RPMI 1640	<b>Growth phase at harvest</b>	Mid log at 1 x 10 <sup>(6)</sup> cells / ml
<b>Wash solution</b>	Phosphate Buffered Saline (PBS)	<b>Pre-pulse incubation</b>	Hepes buffered saline (HBS)

## The Pulse

<b>Electroporation Temperature</b>	Room temperature	<b>Instruments Used</b>	Gene Pulser® apparatus & Capacitance Extender
<b>Electroporation Medium*</b>	Hepes Buffered Saline (HBS)	<b>Cuvette Gap</b>	0.4 cm
<b>Cell Density</b>	5 x 10 <sup>(7)</sup> cells / ml	<b>Voltage</b>	0.32 kV
<b>Volume of Cells</b>	1.0 ml total volume (see notes)*	<b>Field Strength</b>	0.8 kV/cm
<b>DNA Concentration</b>	500 µg / ml total	<b>Capacitor</b>	960 µF
<b>DNA Resuspension Buffer</b>	450 µg / ml carrier + 50 µg / ml plasmid	<b>Resistor</b>	(Pulse Controller) Ω none
<b>Volume of DNA</b>	Not given	<b>Time Constant</b>	12 msec

## After the Pulse

<b>Outgrowth Medium</b>	RPMI 1640	<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>	24 hours	(1.) Margolshee <i>et. al.</i> , <i>Mol. Cell. Biol.</i> <b>8</b> : 2837-2847 (1988). (2.) Canfield <i>et. al.</i> , <i>Mol. Cell. Biol.</i> <b>10</b> : 1367-1372 (1990). (3.) Spickofsky <i>et. al.</i> , <i>DNA and Prot. Eng. Techniques</i> , <b>2</b> : 14-18 (1990).
<b>Selection Method or Assay Used</b>	Hygromycin	*Maximum recommended volume for 0.4 cm cuvettes is 0.8 ml for uniform field strengths.
<b>Electroporation Efficiency</b>	2 x 10 <sup>(4)</sup> to 5 x 10 <sup>(4)</sup>	<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>Per Cent Survival</b>	20 to 30 %	<b>HBS:</b> 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl <sub>2</sub>

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