



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, suspension	Molecules Electroporated	DNA: EBV based vectors, 12-20 kB, closed circular
Species Used	Human, UC729-6, lymphoblastoid, B-cells		

Before the Pulse

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

The Pulse

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

After the Pulse

Outgrowth Medium	RPMI 1640	Relevant Publications and/or Comments
Outgrowth Temperature	37 °C	Note: exponential values designated in parentheses.
Length of Incubation	24 hours	(1.) Margolshee <i>et. al.</i> , <i>Mol. Cell. Biol.</i> 8 : 2837-2847 (1988). (2.) Canfield <i>et. al.</i> , <i>Mol. Cell. Biol.</i> 10 : 1367-1372 (1990). (3.) Spickofsky <i>et. al.</i> , <i>DNA and Prot. Eng. Techniques</i> , 2 : 14-18 (1990).
Selection Method or Assay Used	Hygromycin	*Maximum recommended volume for 0.4 cm cuvettes is 0.8 ml for uniform field strengths.
Electroporation Efficiency	2 x 10 ⁽⁴⁾ to 5 x 10 ⁽⁴⁾	PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH ₂ PO ₄ , 1.15g Na ₂ HPO ₄
Per Cent Survival	20 to 30 %	HBS: 10mM HEPES, pH 7.2, 150 mM NaCl, 5 mM CaCl ₂

Name of Submitter
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