



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, pN2 (retroviral vector), pCDM8-CD34 (both linears and supercoil).
Species Used	Mouse, BbSutA, hematopoietic; Human, K562, HEL, HL 60 (leukemia lines)		

Before the Pulse

Cell growth medium	B6SutA: McCoy's 5A + supplements (15% FCS + 10% WEHI-CM) human lines: RPMI 1640 + 10% calf serum	Growth phase at harvest	Log phase
Wash solution	Dulbecco's PBS, no Ca ⁺⁺ or Mg ⁺⁺	Pre-pulse incubation	5 to 10 min., room temperature

The Pulse

Electroporation Temperature	Room temperature	Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	Dulbecco's PBS, no Ca ⁺⁺ or Mg ⁺⁺		
Cell Density	5 x 10 ⁽⁶⁾ to 10 ⁽⁷⁾ / ml	Cuvette Gap	0.4 cm
Volume of Cells	0.8 ml	Voltage	0.3 kV
DNA Concentration	100 to 1000 ng / µl	Field Strength	0.75 kV/cm
DNA Resuspension Buffer	TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0)	Capacitor	500 µF
Volume of DNA	10 to 50 µl	Resistor	(Pulse Controller) Ω none
		Time Constant	5 to 10 msec

After the Pulse

Outgrowth Medium	B6: McCoy's 5A + supplements, Leukemia lines: RPMI 1640 + 10% calf serum	Relevant Publications and/or Comments	Note: exponential values designated in parentheses.
Outgrowth Temperature	37°C		
Length of Incubation	24 to 48 hours		
Selection Method or Assay Used	G418 (100 to 800 µg / ml); cell survival.		
Electroporation Efficiency	Unknown		
Per Cent Survival	Unknown		

Name of Submitter
Institution Address

Telephone Number
Fax Number
Date Submitted 3/8/91
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