



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: linearized DNA used for stable transfections.
Species Used	Mouse, X-63, myeloma [p3 X63 - Ag8.653]		

Before the Pulse

Cell growth medium	DMEM, 10% Fetal Calf Serum (FCS) (GIBCO/BRL, Sigma)	Growth phase at harvest	50 to 70% confluency
Wash solution	Wash two times in electroporation buffer	Pre-pulse incubation	4° C, 10 min.

The Pulse

Electroporation Temperature	Room temperature	Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	Phosphate Buffered Saline	Cuvette Gap	0.4 cm
Cell Density	5 x 10 ⁽⁵⁾ cells/pulse, stable transfection	Voltage	0.180 kV
Volume of Cells	0.5 ml	Field Strength	0.45 kV/cm
DNA Concentration	10 µg / pulse	Capacitor	960 µF
DNA Resuspension Buffer	Not given; pulse volume: 0.8 ml	Resistor	(Pulse Controller) Ω none
Volume of DNA	Not given; pulse volume: 0.8 ml	Time Constant	24 msec

After the Pulse

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

Name of Submitter	Telephone Number
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