



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	DNA: Bovine papilloma virus E1 in
Species Used	Mouse, C127, fibroblast, mammary tumor	Electroporated	pML2d

Before the Pulse

Cell growth medium	DMEM + 10 % Fetal Calf Serum (GIBCO/BRL, Sigma)	Growth phase at harvest	logarithmic
Wash solution	PBS (Phosphate Buffered Saline) and Electroporation Medium	Pre-pulse incubation	10 minutes on ice in DMEM + 10 % Fetal Calf Serum + BES

The Pulse

Electroporation Temperature	Room temperature	Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	DMEM + 10 % Fetal Calf Serum + BES	Cuvette Gap	0.4 cm
Cell Density	1.5 to 2.0 x10 ⁽⁶⁾ cells/ pulse	Voltage	0.21 kV
Volume of Cells	0.25 ml	Field Strength	0.525 kV/cm
DNA Concentration	10 to 20 µg / pulse	Capacitor	960 µF
DNA Resuspension Buffer	TE (10 mM Tris, 1 mM EDTA, pH 8.0)	Resistor	(Pulse Controller) Ω none
Volume of DNA	25 µl	Time Constant	70 to 80 msec

After the Pulse

Outgrowth Medium	DMEM + 10% Fetal Calf Serum
Outgrowth Temperature	37 °C
Length of Incubation	3 days /transient; 2-3 weeks/ stable
Selection Method or Assay Used	G418 resistance for stable transformation
Electroporation Efficiency	20-30%
Per Cent Survival	30-40 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
PBS: 1x = 8g NaCl, 0.2g KCl, 0.2g KH₂PO₄, 1.15g Na₂HPO₄

Name of Submitter
Institution Address

Telephone Number
Fax Number
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