



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, adherent	Molecules	DNA: pSV2neo, <i>Apa</i> L1 digest.
Species Used	Mouse, LM(TK-), connective tissue [L-M (TK-)].	Electroporated	

Before the Pulse

Cell growth medium	Dulbecco's MEM (GIBCO/BRL, Sigma)	Growth phase at harvest	50 to 75% confluent
Wash solution	Phosphate Buffered Saline, without Ca ⁺⁺ or Mg ⁺⁺	Pre-pulse incubation	None

The Pulse

Electroporation Temperature	4 °C	Instruments Used	Gene Pulser® apparatus
Electroporation Medium*	Phosphate Buffered Saline, without Ca ⁺⁺ or Mg ⁺⁺	Cuvette Gap	0.4 cm
Cell Density	1.25 x 10 ⁽⁷⁾ cells / ml	Voltage	0.950 kV
Volume of Cells	0.8 ml	Field Strength	2.37 kV/cm
DNA Concentration	0.5 µg / ml	Capacitor	25 µF
DNA Resuspension Buffer	Phosphate Buffered sucrose	Resistor	(Pulse Controller) Ω none. NOT RECOMMENDED (SEE
Volume of DNA	10 µl	Time Constant	0.4msec

After the Pulse

Outgrowth Medium	Dulbecco's MEM
Outgrowth Temperature	37 °C
Length of Incubation	48 hours
Selection Method or Assay Used	G-418 , 400 µg / ml after 48 hours recovery
Electroporation Efficiency	1 x 10 ⁽⁻³⁾ transformants / cell
Per Cent Survival	80 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

**It is NOT RECOMMENDED to use high voltage with out the Pulse Controller.

Name of Submitter
Institution Address

Telephone Number
Fax Number
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