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, suspension	**Molecules Electroporated**	DNA: Plasmid DNA, 5 kB -12 kB
Species Used	Human, 293, kidney cells; Hamster, CHO, ovary cells		

Before the Pulse

Cell growth medium	Hamms F-12 / DMEM (50:50) +10% Fetal Bovine Serum (GIBCO/BRL, Sigma)	**Growth phase at harvest**	Log phase
Wash solution	Phosphate Buffered Saline	**Pre-pulse incubation**	Change to Hamms F-12 / DMEM (50:50) media 24 hours prior to pulse.

The Pulse

Electroporation Temperature	Room temperature	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender, Pulse Controller
Electroporation Medium*	High glucose/DMEM (Best we tested)	**Cuvette Gap**	0.4 cm
Cell Density	3 x 10 (6) cells / ml	**Voltage**	0.245 kV
Volume of Cells	800 μl	**Field Strength**	0.098 kV / cm
DNA Concentration	2 μg / ml	**Capacitor**	960 μF
DNA Resuspension Buffer	TE	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	2 μl	**Time Constant**	Not given

After the Pulse

Outgrowth Medium	Hams F-12 / DMEM (50:50) media +10% Fetal Bovine Serum
Outgrowth Temperature	37 °C
Length of Incubation	20 min
Selection Method or Assay Used	G418
Electroporation Efficiency	5%
Per Cent Survival	70 to 80%

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

We are currently testing amplification procedures with linearized plasmid [See: Barsou, M., *DNA and Cell Biology* v.9 (4): 293-300].
PBS: 1x = 8g NaCl,0.2g KCl,0.2g KH_2PO_4, 1.15g Na_2HPO_4
HBS: 10mM HEPES,pH 7.2,150 mM NaCl, 5 mM $CaCl_2$

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