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 Electroporated**	DNA: plasmids of 8.4 kb, linearized
Species Used	Hamster, CHO, ovary		

Before the Pulse

Cell growth medium	DMEM, 10% Fetal Bovine Serum, NEAA, Pennicillin /Streptomycin	**Growth phase at harvest**	Log
		Pre-pulse incubation	10 min on ice
Wash solution	Berg buffer (see Ref in notes)		

The Pulse

Electroporation Temperature	4 °C	**Instruments Used**	Gene Pulser®
Electroporation Medium*	Berg buffer (see Ref. in notes)		
		Cuvette Gap	0.4 cm
Cell Density	10(8) cells /0.8ml	**Voltage**	0 .80 kV
Volume of Cells	0.8 ml	**Field Strength**	2.0 kV/cm
DNA Concentration	20 μg	**Capacitor**	25 μF
DNA Resuspension Buffer	water	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	20 μl	**Time Constant**	0 .6 to 0.8 msec

After the Pulse

Outgrowth Medium	DMEM, 10% FBS, NEAA, Pen/Strep
Outgrowth Temperature	37 °C
Length of Incubation	2 weeks
Selection Method or Assay Used	G418, 8-Aza adenine
Electroporation Efficiency	10 transfectants / μg DNA
Per Cent Survival	20 to 80 % (varies)

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
Ref: Chu,G., Hayakawa, H. and Berg, P.*NAR* **15**, 1131.
Berg buffer: 20 mM HEPES, pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4, 6 mM dextrose.

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