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
Species Used	Mouse, D10.G4.1,T-cell, helper		

Before the Pulse

Cell growth medium	DMEM (10% fetal calf serum) + 20% con A supplement (GIBCO/BRL, Sigma)	**Growth phase at harvest**	log phase
		Pre-pulse incubation	10 to 15 min on ice
Wash solution	Phosphate Buffered Saline without Ca++, Mg++		

The Pulse

Electroporation Temperature	Room Temperature	**Instruments Used**	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	Ca++, Mg++ free		
		Cuvette Gap	0.4 cm
Cell Density	10 (7) cells / 800 μl	**Voltage**	0.3 kV
Volume of Cells	800 μl	**Field Strength**	0.95 kV/cm
DNA Concentration	10 μg in sterile water or TE	**Capacitor**	960 μF
DNA Resuspension Buffer	Not given	**Resistor**	(Pulse Controller) Ω none
Volume of DNA	10 to 20 μl	**Time Constant**	11 msec

After the Pulse

Outgrowth Medium	DMEM (10% serum) + 20% con A supplement
Outgrowth Temperature	37 °C
Length of Incubation	2 days
Selection Method or Assay Used	Hygromycin
Electroporation Efficiency	not quantified (used CAT assay)
Per Cent Survival	70 to 80 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.
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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