



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 Electroported	DNA: linearized (5kB) n-rasCAT plasmids
Species Used	Human, K562, chronic myeloid leukemia.		

Before the Pulse

Cell growth medium	RPMI + 20% Fetal Calf Serum (GIBCO/BRL, Sigma)	Growth phase at harvest	log phase
Wash solution	RPMI	Pre-pulse incubation	None

The Pulse

Electroporation Temperature	Room temperature	Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Electroporation Medium*	RPMI	Cuvette Gap	0.4 cm
Cell Density	10 (7) cells / cuvette	Voltage	2.0 kV
Volume of Cells	850 µl	Field Strength	5 kV/cm
DNA Concentration	50 µg DNA / cuvette	Capacitor	25 µF
DNA Resuspension Buffer	Not given	Resistor	(Pulse Controller) Ω none. NOT RECOMMENDED***(see notes)
Volume of DNA	50 to 300 µl	Time Constant	0.4 msec

After the Pulse

Outgrowth Medium	RPMI + 20% Fetal Calf Serum	Relevant Publications and/or Comments
Outgrowth Temperature	37°C	Note: exponential values designated in parentheses.
Length of Incubation	2 days	It is NOT RECOMMENDED to use high voltage without the Pulse Controller.
Selection Method or Assay Used	G418	
Electroporation Efficiency	Not given	
Per Cent Survival	Not given	

Name of Submitter
Institution Address

Telephone Number
Fax Number
Date Submitted 11/10/90
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