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
Species Used	Human, K562, chronic myeloid leukemia; HeLa, epithelial carcinoma; HEL cells, eythroleukemia.
Molecules Electroporated	DNA: pGL-luciferase vector [Promega]containing β-globin promoter ; co-porated SV 40, β-gal.

Before the Pulse

Cell growth medium	RPMI + 5% Fetal Calf Serum + 5% DCS
Wash solution	Phosphate Bufferd Saline + 5% DCS
Growth phase at harvest	2 to 5 x 10 (5) cells / ml
Pre-pulse incubation	10 min., ice

The Pulse

Electroporation Temperature	25°C
Electroporation Medium*	Hepes Buffered Saline
Cell Density	4 x 10 (7) cells / ml
Volume of Cells	0.5 ml
DNA Concentration	50 μg per pulse
DNA Resuspension Buffer	Not given
Volume of DNA	50 μl
Instruments Used	Gene Pulser® apparatus & Capacitance Extender
Cuvette Gap	0.4 cm
Voltage	0.300 kV
Field Strength	0.75 kV/cm
Capacitor	960 μF
Resistor	(Pulse Controller) Ω none
Time Constant	31 msec

After the Pulse

Outgrowth Medium	RPMI + 5% Fetal Calf Serum + 5% DCS
Outgrowth Temperature	37 °C
Length of Incubation	24 hours
Selection Method or Assay Used	luciferease, β-gal
Electroporation Efficiency	Not given
Per Cent Survival	50 %

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Name of Submittor

Institution Address

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Date Submitted 8/26/92

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