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

Species Used Monkey, COS, kidney cells; Rat, N62 T cells; Mouse, mammary epithelial cells

Molecules Electroporated DNA: linear & supercoiled: Rsv neo, Rsv gal, myc CAT, IRF CAT, fos CAT, hsp CAT, etc.

Before the Pulse

Cell growth medium Fischer's + 10% Horse Serum + 10% Fetal Calf Serum (GIBCO/BRL)

Wash solution

Growth phase at harvest None

Pre-pulse incubation 0 to10 min, room temperature

The Pulse

Electroporation Temperature Room temperature

Electroporation Medium* Hepes buffered saline or Fischer's + 10% Horse Serum +10% Fetal Calf Serum

Cell Density 1 x 10 (7) cells / 0.4 ml

Volume of Cells 0.4 ml to 0.8 ml

DNA Concentration 80 μg/0.3 ml Hepes BufferedSaline

DNA Resuspension Buffer Not given

Volume of DNA < 50 μl

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cuvette Gap 0.4 and 0.2 cm

Voltage 0.25, 0.35, 0.45 kV

Field Strength 625, 875, 1125kV/cm

Capacitor 125, 250, 960 μF

Resistor (Pulse Controller) Ω none

Time Constant 2 to 30 msec

After the Pulse

Outgrowth Medium Fischer's + 10% Horse Serum + 10% Fetal Calf Serum

Outgrowth Temperature Not given

Length of Incubation Not given

Selection Method or Assay Used G418,400 μg / ml

Electroporation Efficiency poor

Per Cent Survival 40 to 50 %

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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