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 and suspension

Species Used Rat, PC12,adrenal pheochromocytoma; Rat brain; Simian (monkey) COS, kidney cells;

Molecules Electroporated DNA: 2 to 4 kB, supercoiled

Before the Pulse

Cell Growth Medium DMEM (GIBCO/BRL, Sigma)

Growth Phase at Harvest Stationary growth

Pre-pulse Incubation 10 min.

Wash Solution Dulbecco's PBS

The Pulse

Instruments Used Gene Pulser® apparatus & Capacitance

Electroporation Temperature 4 °C

Electroporation Medium* Dulbecco's PBS

Cell Density 5 x 10 (6) cells/ ml

Volume of Cells 400 to 800 µl

DNA Concentration 20 to 200 µg / ml

DNA Resuspension Buffer TE (10 mM Tris, 1 mmM EDTA, pH 8.0)

Volume of DNA 10 to 20 µl

Cuvette Gap 0.4 cm

Voltage 0.250 kV

Field Strength 0.625 kV/cm

Capacitor 25 to 500 µF

Resistor (Pulse Controller) Ω none

Time Constant 0.6 to 16 msec

After the Pulse

Outgrowth Medium DMEM

Outgrowth Temperature 37 °C

Length of Incubation days

Selection Method or Assay Used Binding asssays / transients; G418 selection / stable

Electroporation Efficiency varies

Per Cent Survival varies

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

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