



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
Species Used Rat, fibroblasts

Molecules Electroporated DNA, retroviral vector, pN2, 9 kB, linearized at *Hind* III site.

Before the Pulse

Cell growth medium DMEM, 10% Fetal Calf Serum (GIBCO/BRL, Sigma)

Growth phase at harvest 70% confluent

Pre-pulse incubation 2.0 min

Wash solution None

The Pulse

Electroporation Temperature 25 °C
Electroporation Medium* DMEM, 10% Fetal Calf Serum

Instruments Used Gene Pulser® apparatus & Capacitance Extender

Cell Density 10 (7) cells / ml

Cuvette Gap 0.4 cm

Volume of Cells 250 µl

Voltage 0.25 kV

DNA Concentration 0.4 µg / µl

Field Strength 0.625 kV/cm

DNA Resuspension Buffer water

Capacitor 500 µF

Volume of DNA 5 µl

Resistor (Pulse Controller) Ω none

Time Constant 18.0 msec

After the Pulse

Outgrowth Medium DMEM, 10% Fetal Calf Serum

Relevant Publications and/or Comments

Note: exponential values designated in parentheses.

Outgrowth Temperature 37 °C

Length of Incubation 10 days

Selection Method or Assay Used G418 resistance, 500 µl / ml

Electroporation Efficiency 1.21 x 10⁽³⁾ transfectants / µg DNA

Per Cent Survival Not recorded

Name of Submitter
Institution Address

Telephone Number

Fax Number

Date Submitted 8/20/90

Survey Number 148

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